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(54) Title: TUMOR NECROSIS FACTOR FAMILY RECEPTORS AND LIGANDS, ENCODING NUCLEIC ACIDS AND RELATED BINDING AGENTS

(57) Abstract

The invention provides novel receptors of the tumor necrosis factor receptor family as well as ligands of the tumor necrosis factor family. In addition to the isolated receptors and ligands of the invention, there are provided encoding nucleic acids and related selective binding agents.

TUMOR NECROSIS FACTOR FAMILY RECEPTORS AND LIGANDS, ENCODING NUCLEIC ACIDS AND RELATED BINDING AGENTS

BACKGROUND OF THE INVENTION

This invention relates to molecules involved in
5 apoptosis and more specifically to tumor necrosis family
receptors and ligands.

The tumor necrosis factor (TNF) family of
ligands are a superfamily of polypeptides involved in
cell-cell signaling. The prototypic TNF molecule was
10 first identified by its ability to kill a variety of
transformed cell lines. In addition to their role in
cell survival and death, TNF-like molecules and their
cognate receptors are now known to be involved in the
regulation of a variety of cellular functions including
15 cellular proliferation, differentiation and cytokine
secretion.

Ligands of the TNF superfamily are type II
membrane glycoproteins with a large C-terminal
extracellular domain, a single transmembrane spanning
20 region and a variable cytoplasmic domain. Like the
prototypic TNF-α molecule, most are acidic molecules with
approximately 20% sequence homology in the extracellular
receptor-binding domain. The conservation of the more
variable cytoplasmic domains among species homologs
25 indicates that the cytoplasmic domains also can serve an
important function in signal transduction. The TNFR
ligand family includes TNF-α, TNF-β, LTβ, FAS ligand,
CD27 ligand, CD30 ligand, CD40 ligand, OX40 ligand and
4-1BB ligand. Although ligands of the TNF family are
30 typically expressed on the cell surface, soluble forms of
TNF-α, TNF-β and FAS ligand also have been identified.

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TNF receptor family members generally are type
I membrane glycoproteins with sequence homology typically
confined to the extracellular domain. This
amino-terminal extracellular domain includes a variable

5   number of cysteine-rich pseudo repeats, which are motifs
of approximately 40 amino acids containing about six
cysteine residues. Numerous cellular and viral members
of the TNF receptor superfamily have been described,
including TNFR-1 and TNFR-2, TNFR-RP/TNFR-3, nerve growth

10 factor receptor (NGFR), CD27, CD30, CD40, 4-1BB, OX-40,
FAS/APO-1 (CD95), DR3 (Wsl-1/APO-3/TRAMP) and DR4. In
addition, related viral proteins PV-T2 and PV-A53R encode
soluble secreted members of the TNF receptor family.

        Several of the tumor necrosis factor receptors
15 function in the process of regulated cell death, termed
programmed cell death, or "apoptosis." TNFR-1, FAS/APO-1
and DR3 are TNF receptors that transmit a suicidal signal
through a conserved 80 amino acid cytoplasmic region
known as a "death domain." Cytoplasmic death-domain

20 containing proteins including FADD/MORT1 (Fas-associated
protein with death domain) and TRADD (TNFR-1-associated
death domain protein) act as intracellular adaptor
molecules in transmitting the ligand-dependent apoptotic
signal initiated by a "death receptor." The FAS/APO-1

25 receptor appears to directly interact with FADD/MORT1,
while other death receptors such as TNFR-1 and DR3
associate with TRADD, which acts as an adaptor molecule
and recruits FADD/MORT. Despite its sequence homology to
the similar domains present in the death receptors, the

30 death domain of FADD does not induce apoptosis when
over-expressed in mammalian cells and blocks apoptosis
mediated by full-length FADD in a dominant negative
fashion. However, FADD possesses another conserved
domain denoted a "death effector domain" (DED), which

mediates apoptosis when over-expressed in mammalian
cells. Through its DED, FADD binds to homologous domains
present in the cysteine protease (caspase) FLICE/MACH1
(caspase 8). FLICE/MACH1 is the most proximal caspase in
5 a cascade of cysteine proteases that are triggered by
ligand-dependent activation of the death receptors,
ultimating resulting in programmed cell death.

      In addition to mediating apoptosis through
FADD, the TRADD adaptor molecule can recruit other
10 molecules to the aggregated receptor complex of TNFR-1 or
DR3, including the death domain-containing protein RIP
(receptor-interacting protein) and TRAF2, which lacks a
death domain. While recruitment of FADD leads to
activation of caspases and eventual cell death,
15 recruitment of RIP and TRAF2 leads to activation of the
NF-κB (nuclear factor kappa-B) pathway, which may protect
cells from TNF-induced apoptosis.

      Programmed cell death plays a major role
throughout development, as well as in the homeostatic
20 control of cell numbers and in defense against
intracellular pathogens. Furthermore, abnormalities in
programmed cell death contribute to the pathogenesis of a
number of diseases. For example, the failure of cells to
undergo apoptotic cell death can be involved in cancers
25 such as breast, prostate and ovarian cancer, in
autoimmune diseases and in viral infections.
Furthermore, a number of diseases are characterized by
excessive apoptosis including acquired immunodeficiency
syndrome (AIDS); neurodegenerative disorders such as
30 Alzheimer's disease, Parkinson's disease and retinitis
pigmentosa; osteoporosis; ischemic injury; vasculitis;.
hepatic necrosis; and cerebral or myocardial infarction.
Excessive cell death also can contribute to

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organ-specific autoimmune diseases such as Hashimoto
thyroiditis. Therapies which are specifically designed
to modulate apoptotic pathways can change the natural
progression of these and other pathologies characterized
5   by inadequate or excessive apoptosis.

        Unfortunately, the ability to provide effective
therapeutic intervention is limited by the fact that
critical polypeptide receptors and ligands involved in
the process of apoptosis remain to be isolated. For
10 example, novel TNF superfamily receptors, which can play
a critical role in regulating apoptosis in particular
cell types or under certain conditions, remain to be
isolated.

        Thus, there exists a need to identify and
15 isolate novel TNF superfamily receptors and ligands and
their encoding nucleic acids. The present invention
satisfies this need and provides related advantages as
well.

SUMMARY OF THE INVENTION

20   The invention provides novel receptors of the
tumor necrosis factor receptor family as well as ligands
of the tumor necrosis factor family. In addition to the
isolated receptors and ligands of the invention, there
are provided encoding nucleic acids and related selective
25 binding agents.

BRIEF DESCRIPTION OF THE DRAWINGS

        Figure 1. Alignment of the extracellular
domains of several representative tumor necrosis family
receptors. Alignment was done by pileup program of GCG

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software (Genetic Computer Group, Madison, WI).   Dominant
residues are shown in upper case, or determined by the
pretty program of the GCG software.

5   Figure 2.   hAPO8 nucleotide sequence SEQ ID
NO:1 and amino acid sequence SEQ ID NO:2.

         Figure 3.   APO8-related polypeptide (APO8RP)
nucleotide sequence SEQ ID NO:3 and amino acid sequence
SEQ ID NO:4.

         Figure 4.   (A) Alignment of the predicted
10   amino acid sequence of APO8 with APO8RP.   Identical amino
acids are shaded black and homologous residues are shaded
gray.   Predicted signal peptides, transmembrane regions
(TM regions) and death domains are indicated.   (B)
Sequence alignment of the death domains of APO8, DR4,
15   DR3, TNFR-1 and FAS/APO-1.   Residues identical in more
than 30% of sequences are shaded black and those
homologous in greater than 30% of sequences are shaded
gray.   Residues corresponding to the site of the lpr
mutation in the murine FAS receptor are indicated by a Δ
20   symbol.

         Figure 5.   (A) Induction of apoptosis in MCF-7
cells by overexpression of either the full-length APO8
construct or APO8 mutant constructs.   (B) Inhibition of
APO8, APO8RP and DR3 induced apoptosis by progressive
25   increasing amounts of dominant negative FADD (DN-FD) in
293T cells.   The DNA ratio of receptor to DN-FADD is
shown in parenthesis.   © Inhibition of APO8-induced
apoptosis in MCF-7 cells by dominant negative FADD
(DN-FD), dominant negative FLICE (FL-C360S) and the
30   cysteine protease inhibitors CrmA and z-VAD-fmk.
(D) APO8 and APO8RP activate the NF-κB pathway.   Shown is

the relative luciferase activity from an NF-κB luciferase
reporter in 293T cells cotransfected with the indicated
reporter construct.

Figure 6.   hAPO9 nucleotide sequence SEQ ID
5 NO:5 and amino acid sequence SEQ ID NO:6.

Figure 7.   (A)   mAPO4-α long nucleotide
sequence SEQ ID NO:7 and amino acid sequence SEQ ID NO:8.
(B)   mAPO4-α short nucleotide sequence SEQ ID NO:9 and
amino acid sequence SEQ ID NO:10.   © hAPO4-α nucleotide
10 sequence SEQ ID NO:11 and amino acid sequence SEQ ID
NO:12.   (D) rAPO4-α nucleotide sequence SEQ ID NO:13 and
amino acid sequence SEQ ID NO:14.   (E) mAPO4-γ nucleotide
sequence SEQ ID NO:31 and amino acid sequence SEQ ID
NO:32.

15 Figure 8.   mAPO4-β nucleotide sequence SEQ ID
NO:15 and amino acid sequence SEQ ID NO:16.

Figure 9.   hAPO6 nucleotide sequence SEQ ID
NO:17 and amino acid sequence SEQ ID NO:18.

Figure 10. Alignment of representative ligands
20 of the tumor necrosis factor family. Residues identical
in more than 30% of sequences are shaded black and those
homologous in greater than 30% of sequences are shaded
gray.

Figure 11.   (A) hTNRL1-α nucleotide sequence
25 SEQ ID NO:17 and amino acid sequence SEQ ID NO:20.   (B)
mTNRL1-α nucleotide sequence SEQ ID NO:21 and amino acid
sequence SEQ ID NO:22.

Figure 12.   (A) hTNRL1-β nucleotide sequence
SEQ ID NO:23 and amino acid sequence SEQ ID NO:24.   (B)
mTNRL1-β nucleotide sequence SEQ ID NO:25 and amino acid
sequence SEQ ID NO:26.

5   Figure 13.   (A) hTNRL3 nucleotide sequence SEQ
ID NO:27 and amino acid sequence SEQ ID NO:28.   (B)
mTNRL3 nucleotide sequence SEQ ID NO:29 and amino acid
sequence SEQ ID NO:30.

Figure 14.   (A) Induction of apoptosis in 293T
10 cells by expression of full-length human APO4 (hAPO4).
Inhibition of APO4-induced apoptosis by various
inhibitors of the caspase or JNK pathway are also shown.
(B) hAPO4 activates the JNK pathway.   Shown is the
relative luciferase activity from a c-Jun transactivating
15 reporter in 293T cells cotransfected with hAPO4 in the
presence or absence of the indicated inhibitor plasmid
(JBD of JIP-1, MEKK1-D1369A, or DN-TRAF2).   (C) hAPO4
activates the NF-κB pathway.   Shown is the relative
luciferase activity from an NF-κB reporter construct in
20 293T cells cotransfected with hAPO4 and empty vector,
dominant negative TRAF2 (DN-TRAF2) or IκBα-S32/36A.

DETAILED DESCRIPTION OF THE INVENTION

Members of the TNFR family are type I
25 transmembrane receptors characterized by the presence of
cysteine-rich pseudo-repeats in the extracellular domain.
These cysteine rich regions, which are motifs of
approximately 40 amino acids with about 6 cysteines, are
involved in ligand binding.   The average homology in the
30 cysteine-rich extracellular region is in the range of 25%
to 30%.   In addition to a signal peptide, a large
extracellular domain and hydrophobic transmembrane

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region, receptors of the TNF family have cytoplasmic
domains, which function in transmitting a
ligand-dependent signal.

        The TNF receptor superfamily contains at least
5 10 different cellular membrane proteins and several viral
proteins encoding TNF receptor related molecules. The
first cloned receptor of this family was the nerve growth
factor receptor (NGFR), and the TNF receptor superfamily
of type I transmembrane receptors now includes TNFR-1 and
10 TNFR-2, TNFR-RP/TNFR-3, nerve growth factor receptor
(NGFR), CD27, CD30, CD40, 4-1BB, OX-40, FAS/APO-1 (CD95),
DR3 (Wsl-1/APO-3/TRAMP) and DR4. In addition, related
viral proteins PV-T2 and PV-A53R encode soluble secreted
members of the TNF receptor family. Several members such
15 as TNFR-1, TNFR-2 and NGFR have a broad tissue
distribution, while CD27, CD30, CD40, 4-1BB and OX40 are
chiefly restricted to cells of the lymphoid or
hematopoietic systems.

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Table 1				
PERCENTAGE AMINO ACID IDENTITY AND SIMILARITY BETWEEN VARIOUS RECEPTORS IN THE EXTRACELLULAR DOMAIN				
Receptor	hAPO8 (SIN:2)	hAPO9 (SIN:6)	mAPO4-αL (SIN:8)	hAPO6 (SIN:18)
TNFR1	27.3 (46.5)	21.8 (40.0)	20.1 (39.0)	17.0 (32.0)
TNFR2	25.8 (45.1)	21.9 (41.0)	22.4 (41.4)	33.1 (45.0)
Fas	25.1 (45.5)	27.2 (40.9)	23.0 (43.4)	20.4 (38.7)
DR3	21.5 (40.2)	21.6 (36.6)	23.6 (45.9)	28.4 (49.6)
DR4	58.1 (70.6)	57.0 (71.7)	20.4 (36.6)	16.8 (33.7)
* Percentage similarity is shown in parenthesis				

The present invention is directed to the discovery of several new receptors belonging to the TNF family, designated an APO8 polypeptide, an APO8 related polypeptide (APO8RP), an APO9 polypeptide, an APO4 polypeptide, and an APO6 polypeptide. In general, the receptors of the invention are transmembrane proteins with large extracellular ligand-binding domains sharing 16-60% sequence identity in this region with other members of the TNF receptor family (Figure 1 and Table 1 above). A preferred method for determining amino acid identity is by pairwise alignment using the gap program of GCG software (Genetic Computer Group, Madison, WI) using the default parameters.

10

The present invention provides an isolated APO8
polypeptide, which is a novel TNF receptor having
substantially the same amino acid sequence as APO8, or an
active fragment thereof. An isolated APO8 polypeptide of
5   the invention can have substantially the same amino acid
sequence as the APO8 sequence SEQ ID NO:2 shown in
Figure 2.

An exemplary human APO8 polypeptide (hAPO8) is
provided herein. hAPO8 is a widely expressed polypeptide
10 containing structural features reminiscent of known
"death receptors," including an intracellular death
domain. As disclosed herein, overexpression of hAPO8
results in apoptosis, which is caspase-dependent and also
dependent upon the FADD/MORT adaptor molecule
15 (Example II). hAPO8 also activates NF-κB expression
through the TRADD adaptor molecule and, thus, can have
anti-apoptotic activity. As further disclosed herein,
hAPO8 interacts directly with TRADD, thereby recruiting
FADD/MORT. The hAPO8 polypeptide of the invention can be
20 useful in identifying selective enhancers or inhibitors
of APO8 activity, which can be used as novel therapeutics
for the management of APO8-mediated diseases or diseases
involving the dysfunction of programmed cell death. In
addition, nucleic acid molecules encoding hAPO8 or
25 apoptotic fragments of hAPO8 can themselves be used as
inducers or inhibitors of programmed cell death.

The term "isolated," as used herein in
reference to a polypeptide of the invention, means a
polypeptide that is in a form that is relatively free
30 from contaminating lipids, unrelated polypeptides,
nucleic acids and other cellular material normally
associated with the polypeptide in a cell.

As used herein, the term "APO8" means an APO8
polypeptide and includes polypeptides having
substantially the same amino acid sequence as the hAPO8
polypeptide having amino acid sequence SEQ ID NO:2.

5   hAPO8 is a transmembrane protein of 411 amino acids
having the amino acid sequence shown in Figure 2. hAPO8
contains an N-terminal signal peptide of 51 amino acids,
a large extracellular domain with characteristic TNF
receptor family cysteine-rich regions, a hydrophobic

10 transmembrane domain of 27 amino acids (residues 185
to 212) and a C-terminal cytoplasmic domain (residues 213
to 411). The APO8 cytoplasmic domain includes a "death
domain," which is a conserved domain of about 80 amino
acids present in pro-apoptotic TNF receptors such as

15 TNFR-1, FAS/APO-1 and DR3 and that is required for
apoptosis. Death domains also are present in several
cytoplasmic proteins, and a homotypic association between
death domains appears responsible for the interaction of
these cytoplasmic proteins with TNFR-1, FAS/APO-1 and

20 DR3.

As disclosed in Example I, hAPO8 RNA is
expressed in a variety of tissues, with a particularly
high level of expression observed in peripheral blood
lymphocytes (PBL). The results disclosed in Example II

25 demonstrate that the hAPO8 polypeptide of the invention
exhibits caspase-dependent apoptotic activity and
activates NF-κB gene expression. Thus, hAPO8 is a TNF
family receptor, which, like TNFR-1 and DR3, activates
NF-κB expression and promotes apoptosis through a cascade

30 of cysteine proteases.

The term APO8 encompasses a polypeptide having
the sequence of the naturally occurring human APO8
polypeptide (SEQ ID NO:2) and is intended to include

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related polypeptides having substantial amino acid
sequence similarity to hAPO8 (SEQ ID NO:2). Such related
polypeptides exhibit greater sequence similarity to hAPO8
than to other polypeptides containing cysteine-rich
5   pseudo-repeats or other death domain containing
polypeptides and include alternatively spliced forms of
hAPO8, species homologues, and isotype variants of the
amino acid sequence shown in Figure 2. The hAPO8
polypeptide disclosed herein has about 58% amino acid
10 identity and about 71% amino acid similarity with the
Death Receptor (DR4). As used herein, the term APO8
describes polypeptides generally having an amino acid
sequence with greater than about 65% amino acid sequence
identity with hAPO8 (SEQ ID NO:2), preferably greater
15 than about 75% amino acid identity with hAPO8 (SEQ ID
NO:2), more preferably greater than about 85% amino acid
identity with hAPO8 (SEQ ID NO:2), and includes
polypeptides having greater than about 90%, 95% or 97%
amino acid identity with hAPO8 (SEQ ID NO:2).

20   The present invention also provides active
fragments of the APO8 polypeptide of the invention. In
general, the term "active fragment" means a polypeptide
fragment having substantially the same amino acid
sequence as a portion of the indicated polypeptide of the
25 invention, provided that the fragment retains at least
one biological activity of the polypeptide. A portion of
a polypeptide generally has an amino acid sequence of
about 15 to about 400 contiguous residues and can have,
for example, an amino acid sequence of at least about 18,
30 20, 25, 30, 35, 40, 50, 100, 150 or 200 contiguous
residues. As described further below, the invention also
provides active segments of the APO8 polypeptide of the
invention. In general, an active segment is
characterized, in part, by having substantially the same

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amino acid sequence as a portion of the indicated
polypeptide. Such an active segment generally has an
amino acid sequence of about 15 to about 400 contiguous
residues and can have, for example, an amino acid
5 sequence of at least about 18, 20, 25, 30, 35, 40, 50
100, 150 or 200 contiguous residues.

        As used herein, an "active fragment of an APO8
polypeptide" is synonymous with "active fragment of APO8"
or "active APO8 fragment" and means a polypeptide
10 fragment having substantially the same amino acid
sequence as a portion of an APO8 polypeptide, provided
that the fragment retains at least one biological
activity of an APO8 polypeptide. An active fragment of
an APO8 polypeptide can have, for example, an amino acid
15 sequence that is identical or substantially the same as a
portion of the amino acid sequence of hAPO8 (SEQ ID
NO:2), provided that the fragment retains at least one
biological activity of an APO8 polypeptide. A biological
activity of APO8 can be, for example, the ability to bind
20 ligand, bind TRADD or RIP, induce or suppress apoptosis,
activate NF-κB expression, or induce or suppress cell
proliferation, differentiation or cytokine secretion.
Particularly useful active fragment of APO8 are
polypeptide fragments having pro-apoptotic or
25 anti-apoptotic activity. A biological activity of an
APO8 polypeptide or fragment can be routinely assayed;
for example, apoptotic activity can be analyzed by
transfecting an APO8 encoding nucleic acid and measuring
the number of cells with apoptotic morphology, as set
30 forth in Example II.

        An "active fragment of an APO8 polypeptide"
also can be an active APO8 segment, which is a
polypeptide portion having substantially the same amino

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acid sequence as a portion of an APO8 polypeptide,
provided that the segment does not consist of the
identical amino acid sequence encoded by an expressed
sequence tag having GenBank accession number AA223122 or
5   AA232440 and provided that the segment retains at least
one biological activity of an APO8 polypeptide. An
active APO8 segment can have, for example, an amino acid
sequence that is identical or substantially the same as a
portion of the amino acid sequence of hAPO8 (SEQ ID
10   NO:2), provided that the segment does not consist of the
identical amino acid sequence encoded by an expressed
sequence tag having GenBank accession number AA223122 or
AA232440 and provided that the segment retains at least
one biological activity of an APO8 polypeptide.

15   In one embodiment, the invention provides
active fragments that are soluble, extracellularly
expressed forms of the receptors of the invention. Such
a soluble active fragment includes the receptor ligand
binding domain. Such a soluble active fragment can be an
20   inhibitory polypeptide that binds ligand and opposes the
biological function of full-length or membrane-bound
receptor. For example, as disclosed herein, APO8 induces
caspase-dependent apoptosis when expressed in BHK cells;
a soluble form of the APO8 receptor can compete for
25   binding to an APO8 ligand and reduce or inhibit the
pro-apoptotic activity of APO8. Another use of such
soluble forms of the receptors of the invention can be to
transduce a signal across the surface of a cell which
expresses the cognate ligand of the receptor. One
30   skilled in the art understands that a soluble receptor
can act in a systemic fashion, for example, to block the
activity of full-length or membrane-bound receptors
wherever the full-length receptor is expressed.

15

As used herein, the term "soluble" refers to a
polypeptide that is not membrane bound. A particularly
useful soluble polypeptide of the invention is secreted
and, thus, expressed extracellularly.

5   Provided herein is a soluble APO8 active
fragment that includes an APO8 ligand binding domain. A
soluble APO8 active fragment of the invention can be, for
example, a truncated polypeptide encoding the
extracellular domain of APO8. Such a soluble APO8 active
10 fragment can have, for example, substantially the same
amino acid sequence as the signal peptide and
ligand-binding domain of hAPO8 (amino acids 1 to 184 of
hAPO8 shown in Figure 1). One skilled in the art
understands that a soluble APO8 active fragment is
15 distinguished from a membrane-bound fragment by the
deletion or inactivation of the transmembrane domain,
which is shown as amino acids 185 to 212 in Figure 2. A
soluble APO8 active fragment also can be, for example, an
active APO8 segment. Active APO8 segments are described
20 hereinabove.

The present invention also provides an isolated
APO8-related polypeptide, which is a novel TNF
superfamily receptor having substantially the same amino
acid sequence as APO8 or an APO8-related polypeptide. An
25 isolated APO8 related polypeptide of the invention can
have substantially the same amino acid sequence as the
APO8 sequence SEQ ID NO:2 shown in Figure 2 or
substantially the same amino acid sequence as the APO8RP
sequence SEQ ID NO:4 shown in Figure 3. An APO8-related
30 polypeptide is characterized, in part, by the ability to
induce FADD-dependent apoptosis or the ability to
activate NF-κB. As disclosed herein, APO8 and APO8RP
activate NF-κB by a TRADD dependent pathway. As further

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disclosed herein, APO8 and APO8RP interact directly with
TRADD in co-immunoprecipitation experiments, and FADD is
indirectly recruited to APO8 or APO8RP through TRADD. An
APO8-related polypeptide of the invention can be useful
5   in diagnosing an APO8-related polypeptide-mediated
disease or in treating or reducing the severity of such a
disease, as described further below.

        hAPO8 related polypeptides include hAPO8RP, a
polypeptide of 410 amino acids, and hAPO8, a polypeptide
10 of 411 amino acids. hAPO8RP (SEQ ID NO:4) and hAPO8 (SEQ
ID NO:2) share an amino-terminal signal peptide of 51
amino acids with about 53% amino acid identity (see
Figure 4A). An APO8 related polypeptide of the invention
is characterized, in part, in that the amino-terminal 50
15 residues of the mature polypeptide (residues 52 to 101)
have at least about 30% amino acid identity with SEQ ID
NO:2. Although the DR4 sequence described in Pan et al.,
Science 276:111-113 (1997) shares some similarity with
hAPO8 and hAPO8RP, the DR4 sequence has only about 28%
20 amino acid identity in the amino-terminal region of the
mature polypeptide (shown as SEQ ID NO:2). Thus, DR4 is
not an APO8-related polypeptide, as defined herein.

        The term APO8-related polypeptide encompasses a
polypeptide having the sequence of the naturally
25 occurring human APO8 polypeptide (SEQ ID NO:2) or the
naturally occurring APO8-related polypeptide (APO8RP; SEQ
ID NO:4) and is intended to include related polypeptides
having substantial amino acid sequence similarity to
hAPO8 (SEQ ID NO:2) or hAPO8RP (SEQ ID NO:4). Such
30 related polypeptides exhibit greater sequence similarity
to hAPO8 or hAPO8RP than to other polypeptide containing
cysteine-rich pseudo-repeats such as TNFR-1 or DR4 and
include alternatively spliced forms of hAPO8 and hAPO8RP,

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species homologues, and isotype variants of the amino
acid sequences shown in Figures 2 and 3A. As used
herein, the term APO8-related polypeptide describes a
polypeptide generally having an amino acid sequence with
5 greater than about 50% amino acid sequence identity with
hAPO8 (SEQ ID NO:2) and, in particular, having a sequence
in which the amino terminal 50 residues of the mature
polypeptide have at least about 30% identity with amino
acids 52 to 101 of SEQ ID NO:2. An APO8-related
10 polypeptide preferably has an amino acid sequence in
which the amino terminal 50 residues of the mature
polypeptide have at least about 50% amino acid identity
with amino acids 52 to 101 of SEQ ID NO:2, more
preferably greater than about 75% amino acid identity
15 with amino acids 52 to 101 of SEQ ID NO:2, and can be a
polypeptide in which the amino terminal 50 residues of
the mature polypeptide have greater than about 85%, 90%,
95% or 97% amino acid identity with amino acids 52 to 101
of SEQ ID NO:2. One skilled in the art understands that
20 the term APO8-related polypeptide encompasses fusion
proteins, in which a heterologous polypeptide sequence is
fused N- or C- terminally, or is fused internally, for
example, near the cleavage site of the signal peptide.

    The present invention also provides an isolated
25 APO9 polypeptide having substantially the same amino acid
sequence as APO9, or an active fragment thereof. An
isolated APO9 polypeptide of the invention can have
substantially the same amino acid sequence as the human
APO9 sequence SEQ ID NO:6 shown in Figure 6.

30   As used herein, the term "APO9" means an APO9
polypeptide and includes polypeptides having
substantially the same amino acid sequence as the hAPO9
polypeptide having amino acid sequence SEQ ID NO:6. A

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short, alternatively spliced form of hAPO9 having 259
amino acids is shown in Figure 6. This hAPO9 sequence
contains an amino-terminal signal peptide (residues 1 to
22), a large extracellular ligand binding domain with
5 characteristic TNF receptor family cysteine-rich regions
(residues 23 to 241) and a hydrophobic membrane-anchoring
domain (residues 242 to 259) at the 3' end. The hAPO9
extracellular domain has significant homology to other
TNF receptor polypeptides as shown in Figure 1. Human
10 APO9 is most closely related to the DR4 death receptor,
with 57% amino acid sequence identity and 71% amino acid
sequence similarity in the extracellular domain. A stop
codon is present immediately after the membrane-anchoring
domain such that the short form of hAPO9 shown in Figure
15 6 lacks a cytoplasmic domain. Alternatively spliced,
full-length forms of APO9 having a cytoplasmic signaling
domain can mediate apoptosis; their function can be
opposed by the short APO9 form shown in Figure 6.

The term APO9 encompasses a polypeptide having
20 the sequence of the naturally occurring human APO9
polypeptide (SEQ ID NO:6) and is intended to include
related polypeptides having substantial amino acid
sequence similarity to hAPO9 (SEQ ID NO:6). Such related
polypeptides exhibit greater sequence similarity to hAPO9
25 than to members of the TNF receptor superfamily and
include alternatively spliced forms of hAPO9, full-length
forms having a cytoplasmic signaling domain, species
homologues, and isotype variants of the amino acid
sequence shown in Figure 6. Such full-length forms or
30 APO9 species homologues can be readily obtained by the
skilled artisan using routine molecular techniques, for
example, by screening an appropriate cDNA library with a
portion of SEQ ID NO:5 as a probe. As used herein, the
term APO9 describes polypeptides generally having an

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amino acid sequence with greater than about 65% amino
acid sequence identity in the extracellular domain and
transmembrane domain with hAPO9 (SEQ ID NO:6), preferably
greater than about 75% amino acid identity in the
5   extracellular domain and transmembrane domain with hAPO9
(SEQ ID NO:6), more preferably greater than about 85%
amino acid identity in the extracellular domain and
transmembrane domain with hAPO9 (SEQ ID NO:6), and
includes polypeptides having greater than about 90%, 95%
10 or 97% amino acid identity in the extracellular domain
and transmembrane domain with hAPO9 (SEQ ID NO:6).

        The present invention also provides active
fragments of the APO9 polypeptide of the invention. As
used herein, the term "active fragment of an APO9
15 polypeptide" is synonymous with "active fragment of APO9"
or "active APO9 fragment" and means a polypeptide
fragment having substantially the same amino acid
sequence as a portion of an APO9 polypeptide, provided
that the fragment retains at least one biological
20 activity of an APO9 polypeptide. An active fragment of
an APO9 polypeptide can have, for example, an amino acid
sequence that is identical or substantially the same as a
portion of the amino acid sequence of hAPO9 (SEQ ID
NO:6), provided that the fragment retains at least one
25 biological activity of an APO9 polypeptide. A biological
activity of APO9 can be, for example, the ability to bind
ligand, the ability to induce or suppress apoptosis or
the ability to induce or suppress cell proliferation,
differentiation or cytokine secretion. A biological
30 activity of an APO9 polypeptide or fragment can be
routinely assayed; for example, apoptotic activity can be
analyzed by transfecting an APO9 encoding nucleic acid
and measuring the number of cells with apoptotic
morphology, as set forth in Example II.

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An "active fragment of an APO9 polypeptide" can be an active APO9 segment, which is a polypeptide portion having substantially the same amino acid sequence as a portion of an APO9 polypeptide, provided that the segment

5   does not consist of the identical amino acid sequence by an expressed sequence tag having GenBank accession number AA031883, AA150849, T71406 or R10995 and provided that the segment retains at least one biological activity of an APO9 polypeptide. An active APO9 segment can have,

10 for example, an amino acid sequence that is identical or substantially the same as a portion of the amino acid sequence of hAPO9 .(SEQ ID NO:6), provided that the segment does not consist of the identical amino acid sequence encoded by an expressed sequence tag having

15 GenBank accession number AA031883, AA150849, T71406 or R10995 and provided that the segment retains at least one biological activity of an APO9 polypeptide.

.In one embodiment, the invention provides a soluble APO9 active fragment that includes an APO9 ligand

20 binding domain. A soluble APO9 active fragment of the invention can be, for example, a truncated polypeptide encoding the extracellular domain of APO9. An exemplary soluble APO9 active fragment is provided in Figure 1 as amino acids 1 to 241 of hAPO9 and includes the signal

25 peptide and ligand binding domain. One skilled in the art understands that a soluble APO9 active fragment is distinguished from a membrane-bound fragment of APO9 by the deletion or inactivation of the hydrophobic membrane-anchoring domain, shown as amino acids 242 to

30 259 in Figure 6. A soluble APO9 active fragment also can be, for example, an active APO9 segment. Active APO9 segments are described hereinabove.

The present invention also provides an isolated
APO4 polypeptide having substantially the same amino acid
sequence as an APO4 polypeptide, or an active fragment
thereof. An isolated APO4 polypeptide of the invention
5   can have substantially the same amino acid sequence as
murine APO4-αL (SEQ ID NO:8) shown in Figure 7A, murine
APO4-αS (SEQ ID NO:10) shown in Figure 7B, human APO4-α
(SEQ ID NO:12) shown in Figure 7C, or rat APO4-α (SEQ ID
NO:14) shown in Figure 7D. An isolated APO4 polypeptide
10 of the invention also can have substantially the same
amino acid sequence as murine APO4-β (SEQ ID NO:16) as
shown in Figure 8.

As used herein, the term "APO4 polypeptide"
means an APO4-α or APO4-β polypeptide and includes
15 polypeptides having substantially the same amino acid
sequence as the mAPO4-αL polypeptide (SEQ ID NO:8), the
mAPO4-αS polypeptide (SEQ ID NO:10), the hAPO4-α
polypeptide (SEQ ID NO:12), the rAPO4-α polypeptide (SEQ
ID NO:14) or the mAPO4-β polypeptide (SEQ ID NO:16).
20 Murine APO4-α is an alternatively spliced protein
including an amino-terminal signal peptide, cysteine-rich
pseudo-repeats characteristic of the TNF receptor family
and a highly hydrophobic stretch of amino acids
representing a transmembrane domain. The short mAPO4-α
25 isoform (mAPO4-αS) is encoded by an open reading frame of
214 amino acids and includes a short cytoplasmic tail,
while the long mAPO4-αL isoform (mAPO4-αL) is a
polypeptide of 416 amino acids with a unique cytoplasmic
tail having no significant homology to other members of
30 the TNF receptor family. The truncated mAPO4-αS
polypeptide can compete for binding to an APO4-α ligand,
thereby opposing the function of a long form of APO4-α
such as mAPO4-αL.

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Human APO4-α (hAPO4-α) is another APO4
polypeptide of the invention. The nucleotide sequence
encoding hAPO4-α is shown in Figure 7C. This sequence
encodes an hAPO4-α polypeptide of 423 amino acids with
5   70.7% amino acid identity and 81.1% amino acid similarity
to mAPO4-α. As shown in Figure 7C, the hAPO4-α
polypeptide possesses a signal peptide (residues 1 to
23), a putative transmembrane domain (residues 178 to
191) and the characteristic cytoplasmic domain of APO4
10   polypeptides. Rat APO4-α (rAPO4-α) is another APO4
polypeptide of the invention. The available sequence of
the rAPO4-α cDNA is shown in Figure 7D. The cytoplasmic
tail of rAPO4-α contains the unique APO4 cytoplasmic
domain and is 80% identical with mAPO4-α at the amino
15   acid level. Murine APO4-β (mAPO4-β) is another APO4
polypeptide of the invention; the available sequence of
mAPO4-β is shown in Figure 8. Finally, murine APO4-γ
(mAPO4-γ) is another APO4 peptide of the invention whose
sequence is shown in Figure 7E. mAPO4-γ lacks a
20   transmembrane domain and therefore represents a soluble
receptor which can compete for binding to the APO4
ligand, thereby opposing the function of the long forms
of mAPO4 such as mAPO4-αL.

     The term APO4 polypeptide encompasses a
25   polypeptide having the sequence of naturally occurring
murine APO4-αL (SEQ ID NO:8) or APO4-αS (SEQ ID NO:10),
naturally occurring human APO4-α (SEQ ID NO:12) or rat
APO4-α (SEQ ID NO:14) or naturally occurring murine
APO4-β (SEQ ID NO:16) or naturally occurring murine APO4-
30   γ (SEQ ID NO:32) and is intended to include related
polypeptides having substantial amino acid sequence
similarity to SEQ ID NOS:8, 10, 12, 14, 16 or 32. Such
related polypeptides exhibit greater sequence similarity
to mAPO4-αL, mAPO4-αS, hAPO4-α, rAPO4-α, mAPO4-β or

mAPO4-γ than to other TNF family receptors and include
alternatively spliced forms of human, murine or rat
APO4-α, APO4-β or APO4-γ full-length forms of APO4,
species homologues, and isotype variants of the amino
5 acid sequences shown in Figures 7 and 8. As used herein,
the term APO4 polypeptide describes polypeptides
generally including an amino acid region with greater
than about 35% amino acid sequence identity with mAPO4-αL
(SEQ ID NO:8), mAPO4-αS (SEQ ID NO:10), hAPO4-α (SEQ ID
10 NO:12), rAPO4-α (SEQ ID NO:14) or mAPO4-β (SEQ ID NO:16).
In particular, an APO4 polypeptide can have greater than
about 55% amino acid identity, preferably greater than
about 65% amino acid identity, more preferably greater
than about 75% amino acid identity, still more preferably
15 greater than about 85% amino acid identity and most
preferably greater than about 90%, 95% or 97% amino acid
identity with mAPO4-αL (SEQ ID NO:8), mAPO4-αS (SEQ ID
NO:10), hAPO4-α (SEQ ID NO:12), rAPO4-α (SEQ ID NO:14),
mAPO4-β (SEQ ID NO:16) or mAPO4-γ (SEQ ID NO:32).

20   The present invention also provides active
fragments of the APO4 polypeptides of the invention. As
used herein, the term "active fragment of an APO4
polypeptide" is synonymous with "active fragment of APO4"
or "active APO4 fragment" and means a polypeptide
25 fragment having substantially the same amino acid
sequence as a portion of an APO4-α, APO4-β or APO4-γ
polypeptide, provided that the fragment retains at least
one biological activity of an APO4 polypeptide. An
active fragment of an APO4 polypeptide can have, for
30 example, an amino acid sequence that is identical or
substantially the same as a portion of the amino acid
sequence of mAPO4-αL (SEQ ID NO:8), mAPO4-αS (SEQ ID
NO:10), hAPO4-α (SEQ ID NO:12), rAPO4-α (SEQ ID NO:14),
mAPO4-β (SEQ ID NO:16), or APO4-γ (SEQ ID NO:32),

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provided that the fragment retains at least one
biological activity of an APO4 polypeptide. A biological
activity of an APO4 polypeptide can be, for example, the
ability to bind an APO4 ligand, the ability to induce or
5 suppress apoptosis, the ability to activate the JNK
pathway, or the ability to induce or suppress cell
proliferation, differentiation or cytokine secretion. A
biological activity of an APO4 polypeptide or fragment
can be routinely assayed; for example, apoptotic activity
10 can be analyzed by transfecting an APO4 encoding nucleic
acid and measuring the number of cells with apoptotic
morphology, as set forth in Example II.

     An active fragment of APO4 also be, for
example, a cytoplasmic fragment of APO4. An active
15 fragment of APO4 can be an N-terminal fragment having the
amino terminal 355 amino acids of APO4, which was able to
activate the JNK pathway in 293 EBNA cells.

     An "active fragment of an APO4 polypeptide"
also can be an active segment of an APO4 polypeptide.
20 Such an active segment is a polypeptide portion having
substantially the same amino acid sequence as a portion
of an APO4 polypeptide, provided that the segment does
not consist of the identical amino acid sequence encoded
by an expressed sequence tag having GenBank accession
25 number AA036247, AA003356, W55289, AA445805 or W56629 and
provided that the segment retains at least one biological
activity of an APO4 polypeptide. An active APO4
polypeptide segment can have, for example, an amino acid
sequence that is identical or substantially the same as a
30 portion of the amino acid sequence of mAPO4-αL (SEQ ID
NO:8), mAPO4-αS (SEQ ID NO:10), hAPO4-α (SEQ ID NO:12),
rAPO4-α (SEQ ID NO:14), mAPO4-β (SEQ ID NO:16) or APO4-γ
(SEQ ID NO:32), provided that the segment does not

consist of the identical amino acid sequence encoded by
an expressed sequence tag having GenBank accession number
AA036247, AA003356, W55289, AA445805 or W56629 and
provided that the segment retains at least one biological
5 activity of an APO4 polypeptide.

       In one embodiment, the invention provides a
soluble active fragment of an APO4 polypeptide. Such a
soluble active fragment includes the ligand binding
domain of an APO4 polypeptide and can be, for example, a
10 truncated polypeptide encoding the extracellular domain
of an APO4 polypeptide. A soluble active fragment of an
APO4 polypeptide can have, for example, substantially the
same amino acid sequence as the signal peptide and
ligand-binding domain of mAPO4-α (amino acids 1 to 177 of
15 mAPO4-α shown in Figure 7A) or substantially the same
sequence as mAPO4-γ (Figure 7E) or substantially the same
sequence as the signal peptide and ligand-binding domain
of hAPO4-α (amino acids 1 to 177 of hAPO4-α shown in
Figure 7C). A specific example of such a soluble
20 fragment is mAPO4-γ. One skilled in the art understands
that a soluble active fragment of an APO4 polypeptide is
distinguished from a membrane-bound form by deletion or
inactivation of the transmembrane domain. The
transmembrane domain of murine APO4-α is shown in Figure
25 7A as amino acids 173 to 191, and the transmembrane
domain of human APO4-α is shown in Figure 7C as amino
acids 178 to 191. A soluble active fragment of an APO4
polypeptide also can be, for example, an active segment
of an APO4 polypeptide. Such active segments of APO4
30 polypeptides are described hereinabove.

       In another embodiment, the invention provides
an APO4-related polypeptide, which has a sequence with
substantial similarity to the unique cytoplasmic domain

of an APO4 polypeptide. The APO4 cytoplasmic domain is a
conserved region that can function to promote
self-association or association with a different protein.
The APO4 cytoplasmic domain also can promote
5 ligand-dependent cell survival, proliferation,
differentiation or death. An APO4-related polypeptide
can be, for example, a transmembrane receptor including a
cytoplasmic domain having substantial similarity to the
cytoplasmic domain of mAPO4-αL or hAPO4-α. The
10 extracellular domain of an APO4-related polypeptide
receptor can be substantially the same as, or can be
entirely different than the extracellular domain of an
APO4 polypeptide. APO4-related polypeptides also include
intracellularly expressed polypeptides that contain one
15 or more APO4 cytoplasmic domains. An APO4 cytoplasmic
domain can have, for example, the amino acid sequence
shown as amino acids 192 to 416 in Figure 7A, the amino
acid sequence shown as amino acids 192 to 423 in Figure
7C, or an amino acid sequence with substantial similarity
20 to one of these sequences.

        The invention further provides an isolated APO6
polypeptide having substantially the same amino acid
sequence as APO6, or an active fragment thereof. An
isolated APO6 polypeptide of the invention can have, for
25 example, substantially the same amino acid sequence as
the human APO6 sequence SEQ ID NO:18 shown in Figure 9.

        As used herein, the term "APO6" means an APO6
polypeptide and includes polypeptides having
substantially the same amino acid sequence as the hAPO6
30 polypeptide having amino acid sequence SEQ ID NO:18. A
partial nucleotide sequence encoding hAPO6, which lacks
5' and 3' sequence, is shown in Figure 9. The available
hAPO6 sequence shows about 33% homology to the

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extracellular domain of TNFR-2. The partial APO6
sequence shown in Figure 9 can be part of a full-length
APO6 transmembrane receptor.

The term APO6 encompasses a polypeptide having
5   the sequence of the naturally occurring human APO6
polypeptide (SEQ ID NO:18) and is intended to include
related polypeptides having substantial amino acid
sequence similarity to hAPO6 (SEQ ID NO:18). Such
related polypeptides exhibit greater sequence similarity
10 to hAPO6 than to other members of the TNF receptor
superfamily and include alternatively spliced forms of
hAPO6, full-length forms of APO6 having a cytoplasmic
signaling domain, species homologues, and isotype
variants of the amino acid sequence shown in Figure 9.
15 Such full-length forms or APO6 species homologues can be
readily obtained by the skilled artisan using routine
molecular techniques, for example, by screening an
appropriate cDNA library with a portion of SEQ ID NO:17
as a probe. As used herein, the term APO6 describes
20 polypeptides generally having an amino acid sequence with
greater than about 40% amino acid identity with the
extracellular domain of hAPO6 (SEQ ID NO:18), preferably
greater than about 65% amino acid identity with the
extracellular domain of hAPO6 (SEQ ID NO:18), more
25 preferably greater than about 75% amino acid identity
with the extracellular domain of hAPO6 (SEQ ID NO:18),
still more preferably greater than about 75% amino acid
identity with the extracellular domain of hAPO6 (SEQ ID
NO:18), and most preferably about 90%, 95% or 97% amino
30 acid identity with the extracellular domain of hAPO6 (SEQ
ID NO:18).

The present invention also provides active
fragments of the APO6 polypeptide of the invention. As

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used herein, the term "active fragment of an APO6
polypeptide" is synonymous with "active fragment of APO6"
or "active APO6 fragment" and means a polypeptide
fragment having substantially the same amino acid
5   sequence as a portion of an APO6 polypeptide, provided
that the fragment retains at least one biological
activity of an APO6 polypeptide. An active fragment of
an APO6 polypeptide can have, for example, an amino acid
sequence that is identical or substantially the same as a
10 portion of an amino acid sequence of hAPO6 (SEQ ID
NO:18), provided that the fragment retains at least one
biological activity of an APO6 polypeptide. Biological
activities of APO6 include the ability to bind an APO6
ligand, the ability to induce or suppress apoptosis and
15 the ability to induce or suppress cell proliferation,
differentiation or cytokine secretion. A biological
activity of an APO6 polypeptide or fragment can be
routinely assayed; for example, apoptotic activity can be
analyzed by transfecting an APO6 encoding nucleic acid
20 and measuring the number of cells with apoptotic
morphology, as set forth in Example II.

An active fragment of an APO6 polypeptide also
can be an active APO6 segment, which is a polypeptide
segment having substantially the same amino acid sequence
25 as a portion of an APO6 polypeptide, provided that the
segment does not consist of the identical amino acid
sequence encoded by an expressed sequence tag having
GenBank accession number AA025673, AA025672, AA155701,
AA155646, W67560 or AA158406 and provided that the
30 segment retains at least one biological activity of an
APO6 polypeptide. An active APO6 segment can have, for
example, an amino acid sequence that is identical or
substantially the same as a portion of the amino acid
sequence of hAPO6 (SEQ ID NO:18), provided that the

segment does not consist of the identical amino acid
sequence encoded by an expressed sequence tag having
GenBank accession number AA025673, AA025672, AA155701,
AA155646, W67560 or AA158406 and provided that the
5   segment retains at least one biological activity of an
APO6 polypeptide.

        In one embodiment, the invention provides a
soluble APO6 active fragment that includes an APO6 ligand
binding domain. A soluble APO6 active fragment can be,
10 for example, a truncated polypeptide encoding the
extracellular domain of an APO6 polypeptide. A soluble
APO6 active fragment can have, for example, substantially
the same amino acid sequence as amino acids 1 to 168 of
the hAPO6 sequence shown in Figure 1. Soluble APO6
15 active fragments are distinguished from membrane-bound
forms by the absence of a functional transmembrane
domain. A soluble APO6 active fragment also can be, for
example, an active APO6 segment. Active APO6 segments
are described hereinabove.

20   Ligands of the TNF ligand superfamily are
acidic, TNF-like molecules with approximately 20%
sequence homology in the extracellular receptor-binding
domain. Most exist principally as type II membrane
glycoproteins, with the biologically active form a
25 trimeric or multimeric complex. Like the prototypic
TNF-α molecule, most have a large C-terminal
extracellular domain, a single transmembrane spanning
region and a variable amino-terminal cytoplasmic domain.
The TNFR ligand family includes TNF-α, TNF-β, LTβ, FAS
30 ligand, CD27 ligand, CD30 ligand, CD40 ligand, 4-1BB
ligand, OX40 ligand and FAS/APO-1 ligand. Although
members of the TNF ligand superfamily typically are cell

surface-expressed molecules, soluble forms of TNF-α,
TNF-β and FAS ligand also have been identified.

Disclosed herein is subfamily of polypeptide
ligands designated the tumor necrosis factor related
5   ligand 1 (TNRL1) subfamily. Thus, the present invention
provides an isolated TNRL1 polypeptide having
substantially the same amino acid sequence as TNRL1, or
an active fragment thereof. An isolated TNRL1
polypeptide of the invention can have, for example,
10 substantially the same amino acid sequence as the
sequence of human TNRL1-α (SEQ ID NO:20); murine TNRL1-α
(SEQ ID NO:22); human TNRL1-β (SEQ ID NO:24) or murine
TNRL1-β (SEQ ID NO:26).

As used herein, the term "TNRL1" means a TNRL1
15 polypeptide and includes polypeptides having
substantially the same amino acid sequence as the
hTNRL1-α polypeptide having amino acid sequence SEQ ID
NO:20; the mTNRL1-α polypeptide having amino acid
sequence SEQ ID NO:22; the hTNRL1-β polypeptide having
20 amino acid sequence SEQ ID NO:24; and the mTNRL1-β
polypeptide having amino acid sequence SEQ ID NO:26.
Human TNRL1-α (hTNRL1-α) is a polypeptide of at least
230(?) amino acids; the available hTNRL1-α nucleotide
(SEQ ID NO:19) and amino acid sequence (SEQ ID NO:20) are
25 shown in Figure 11A. Human TNRL1-α (SEQ ID NO:20)
exhibits about 25% amino acid identity and about 49%
amino acid similarity to TNF-α.

Murine TNRL1-α (mTNRL1-α) is the homolog of
human TNRL1-α and also is an TNRL1 polypeptide of the
30 invention. mTNRL1-α is a polypeptide of at least 290
amino acids; the available mTNRL1-α nucleotide (SEQ ID
NO:21) and amino acid sequence (SEQ ID NO:22) are shown

in Figure 11B. Murine TNRL1-α (SEQ ID NO:22) is about
68% identical and 81% similar to human TNRL1-α at the
amino acid level.

          Human TNRL1-β (hTNRL1-β) also is a TNRL1
5   polypeptide of the invention. Human TNRL1-β is a
polypeptide of 250 amino acids; the hTNRL1-β nucleotide
sequence (SEQ ID NO:23) and amino acid sequence (SEQ ID
NO:24) are shown in Figure 12A. The hTNRL1-β polypeptide
(SEQ ID NO:24) is about 31% identical and 55% similar to
10 hTNRL1-α at the amino acid level and is more distantly
related to TNF-α (see Figure 10).

          Murine TNRL1-β (mTNRL1-β), the homolog of human
TNRL1-β, also is a TNRL1 polypeptide of the invention.
The nucleotide (SEQ ID NO:25) and corresponding amino
15 acid sequence (SEQ ID NO:26) of full-length mTNRL1-β are
shown in Figure 12B.

          The term TNRL1 encompasses a polypeptide having
the sequence of the naturally occurring human TNRL1-α
polypeptide SEQ ID NO:20, the murine TNRL1-α polypeptide
20 SEQ ID NO:22, the human TNRL1-β polypeptide SEQ ID NO:24,
or the murine TNRL1-β polypeptide SEQ ID NO:26 and is
intended to include related polypeptides having
substantial amino acid sequence similarity to hTNRL1-α,
mTNRL1-α, hTNRL1-β or mTNRL1-β. Such related
25 polypeptides exhibit greater sequence similarity to
hTNRL1-α, mTNRL1-α, hTNRL1-β or mTNRL1-β than to other
members of the TNF ligand superfamily, such as TNF-α, and
include alternatively spliced forms of hTNRL1-α, full-
length forms of hTNRL1-α, mTNRL1-α, hTNRL1-β or mTNRL1-β,
30 species homologues, and isotype variants of the amino
acid sequences shown in Figures 11 and 12. As used
herein, the term TNRL1 describes polypeptides generally

having an amino acid sequence with greater than about 30%
amino acid identity with hTNRL1-α (SEQ ID NO:20),
mTNRL1-α (SEQ ID NO:22), hTNRL1-β (SEQ ID NO:24) or
mTNRL1-β (SEQ ID NO:26), preferably greater than about
5   35% amino acid identity with hTNRL1-α (SEQ ID NO:20),
mTNRL1-α (SEQ ID NO:22), hTNRL1-β (SEQ ID NO:24) or
mTNRL1-β (SEQ ID NO:26), more preferably greater than
about 45% amino acid identity with hTNRL1-α (SEQ ID
NO:20), mTNRL1-α (SEQ ID NO:22), hTNRL1-β (SEQ ID NO:24)
10   or mTNRL1-β (SEQ ID NO:26), and most preferably about
65%, 75%, 85%, 90%, 95% or 97% amino acid identity with
hTNRL1-α (SEQ ID NO:20), mTNRL1-α (SEQ ID NO:22),
hTNRL1-β (SEQ ID NO:24) or mTNRL1-β (SEQ ID NO:26).

        The present invention also provides an isolated
15   TNRL1 polypeptide having substantially the same amino
acid sequence as TNRL1, or an active fragment of a TNRL1
polypeptide of the invention. An isolated TNRL1
polypeptide of the invention can have, for example,
substantially the same amino acid sequence as the human
20   TNRL1 (SEQ ID NO:20) shown in Figure 11.

        The present invention also provides active
fragments of a TNRL1 polypeptide of the invention. As
used herein, the term "active fragment of an TNRL1
polypeptide" is synonymous with "active fragment of
25   TNRL1" or "active TNRL1 fragment" and means a polypeptide
fragment having substantially the same amino acid
sequence as a portion of a TNRL1 polypeptide, provided
that the fragment retains at least one biological
activity of a TNRL1 polypeptide. An active fragment of a
30   TNRL1 polypeptide can have, for example, an amino acid
sequence that is identical or substantially the same as a
portion of the amino acid sequence of hTNRL1-α (SEQ ID
NO:20), mTNRL1-α (SEQ ID NO:22), hTNRL1-β (SEQ ID NO:24)

or mTNRL1-β (SEQ ID NO:26), provided that the fragment retains at least one biological activity of a TNRL1 polypeptide. A biological activity of a TNRL1 polypeptide can be, for example, the ability to bind a
5   TNRL1 receptor, the ability to induce or suppress apoptosis or the ability to induce or suppress cell proliferation, differentiation or cytokine secretion. A biological activity of a TNRL1 polypeptide or fragment can be routinely assayed; for example, apoptotic activity
10 can be analyzed by treating BJAB cells with polypeptide and assaying cell survival as set forth in Example VI.

An "active fragment of a TNRL1 polypeptide" also can be an active TNRL1 segment, which is a polypeptide segment having substantially the same amino
15 acid sequence as a portion of a TNRL1 polypeptide, provided that the segment does not consist of the identical amino acid sequence encoded by an expressed sequence tag having GenBank accession number AA166695, T87299, R16882, AA254047, AA056924, AA057069, N91002,
20 N79018 or AA497494 or TIGR sequence number M78231 and provided that the segment retains at least one biological activity of a TNRL1 polypeptide. An active TNRL1 segment can have, for example, an amino acid sequence that is identical or substantially the same as a portion
25 of the amino acid sequence of hTNRL1-α (SEQ ID NO:20), mTNRL1-α (SEQ ID NO:22), hTNRL1-β (SEQ ID NO:24) or mTNRL1-β (SEQ ID NO:26), provided that the segment does not consist of the identical amino acid sequence encoded by an expressed sequence tag having GenBank accession
30 number AA166695, T87299, R16882, AA254047, AA056924, AA057069, N91002, N79018 or AA497494 or TIGR sequence number M78231 and provided that the segment retains at least one biological activity of a TNRL1 polypeptide.

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        In one embodiment, the invention provides a
soluble active fragment of a TNRL1 ligand of the
invention, where the active fragment includes the TNRL1
receptor binding domain. A TNRL1 ligand of the invention
5 can be, for example, a membrane-bound ligand that
transduces a signal when the cell on which it is
expressed contacts a cell bearing its cognate receptor
or, alternatively, a soluble form of TNRL1 can be
expressed extracellularly and bind a distant
10 receptor-bearing cell. Such a soluble TNRL1 active
fragment can have the activity of a full-length TNRL1 of
the invention or can be an inhibitory polypeptide that
opposes the biological function of full-length ligand.

        Thus, the invention provides a soluble TNRL1
15 active fragment that includes a TNRL1 receptor binding
domain. A soluble TNRL1 active fragment can be, for
example, a truncated polypeptide encoding the
extracellular domain of a TNRL1 polypeptide and can have,
for example, an amino acid sequence that is identical or
20 substantially the same as amino acids 22 to 225 of
hTNRL1-α, amino acids 32 to 243 of mTNRL1-α, amino acids
50 to 250 of hTNRL1-β or amino acids 42 to 241 of
mRNRL-β, shown in Figure 10. One skilled in the art
understands that soluble TNRL1 active fragments lack a
25 functional TNRL1 transmembrane domain. A soluble TNRL1
active fragment also can be an active TNRL1 segment.
Segments having an amino acid sequence identical to the
amino acid sequence encoded by an expressed sequence tag
having GenBank accession number AA166695, T87299, R16882,
30 AA254047, AA056924, AA057069, N91002, N79018 or AA497494
or TIGR sequence number M78231 are excluded from the
definition of an active TNRL1 segment, as defined herein.

35

The present invention further provides an
isolated TNRL3 polypeptide having substantially the same
amino acid sequence as TNRL3, or an active fragment
thereof. An isolated TNRL3 polypeptide of the invention

5   can have, for example, substantially the same amino acid
sequence as the human TNRL3 (SEQ ID NO:28) or murine
TNRL3 (SEQ ID NO:30) sequence shown in Figure 13.

As used herein, the term "TNRL3" means a TNRL3
polypeptide and includes polypeptides having

10   substantially the same amino acid sequence as the hTNRL3
polypeptide having amino acid sequence SEQ ID NO:28 or
the mTNRL3 polypeptide having amino acid sequence SEQ ID
NO:30. A partial nucleotide sequence encoding the 3'
portion of hTNRL3 is shown in Figure 13A. The available

15   sequence shows homology to TRAIL ligand and to other
members of the TNF ligand superfamily (see Figure 10).
The murine TNRL3 homolog, mTNRL3, also is a TNRL3
polypeptide of the invention. The partial nucleotide and
amino acid sequence of murine TNRL3 lacks some 5'

20   sequence; the available sequence is shown in Figure 13B.

The term TNRL3 encompasses a polypeptide having
the sequence of naturally occurring human TNRL3 (SEQ ID
NO:28) or murine TNRL3 (SEQ ID NO:30) and is intended to
include related polypeptides having substantial amino

25   acid sequence similarity to the human or murine TNRL3
polypeptides SEQ ID NOS:28 or 30. Such related
polypeptides exhibit greater sequence similarity to
hTNRL3 or mTNRL3 than to other members of the TNF ligand
superfamily such as TRAIL and include alternatively

30   spliced forms of hTNRL3 or mTNRL3, species homologues,
full-length forms and isotype variants of the amino acid
sequences shown in Figure 13. As used herein, the term
TNRL3 describes polypeptides generally having an amino

acid sequence with greater than about 30% amino acid
sequence identity with hTNRL3 or mTNRL3 (SEQ ID NO:28 or
SEQ ID NO:30), preferably greater than about 35% amino
acid identity with hTNRL3 or mTNRL3, more preferably
5   greater than about 45% amino acid identity with hTNRL3 or
mTNRL3 (SEQ ID NO:28 or SEQ ID NO:30), still more
preferably greater than about 65% amino acid identity
with hTNRL3 or mTNRL3 (SEQ ID NO:28 or SEQ ID NO:30), and
most preferably about 75%, 85%, 90%, 95% or 97% amino
10   acid identity with hTNRL3 or mTNRL3 (SEQ ID NO:28 or SEQ
ID NO:30).

        Further provided herein is an active fragment
of a TNRL3 polypeptide of the invention. As used herein,
the term "active fragment of an TNRL3 polypeptide" is
15   synonymous with "active fragment of TNRL3" or "active
TNRL3 fragment" and means a polypeptide fragment having
substantially the same amino acid sequence as a portion
of a TNRL3 polypeptide, provided that the fragment
retains at least one biological activity of a TNRL3
20   polypeptide. An active fragment of a TNRL3 polypeptide
can have, for example, substantially the same amino acid
sequence as a portion of hTNRL3 (SEQ ID NO:28) or mTNRL3
(SEQ ID NO:30), provided that the fragment retains at
least one biological activity of a TNRL3 polypeptide. A
25   biological activity of a TNRL3 polypeptide can be, for
example, the ability to bind a TNRL3 receptor, the
ability to induce or suppress apoptosis or the ability to
induce or suppress cell proliferation, differentiation or
cytokine secretion. A biological activity of a TNRL3
30   polypeptide or fragment can be routinely assayed; for
example, apoptotic activity can be analyzed by treating
BJAB cells with polypeptide and assaying cell survival as
set forth in Example VI.

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An "active fragment of a TNRL3 polypeptide"
also can be an active TNRL3 segment, which is a
polypeptide segment having substantially the same amino
acid sequence as a portion of a TNRL3 polypeptide,
5   provided that the segment does not consist of the amino
acid sequence, or a segment thereof, encoded by an
expressed sequence tag having GenBank accession number
R55285, N35070, C00994 or AA221610 and provided that the
segment retains at least one biological activity of a
10 TNRL3 polypeptide. An active TNRL3 segment can have, for
example, an amino acid sequence that is identical or
substantially the same as a portion of the amino acid
sequence of hTNRL3 (SEQ ID NO:28) or mTNRL3 (SEQ ID
NO:30), provided that the segment does not consist of the
15 amino acid sequence, or a segment thereof, encoded by an
expressed sequence tag having GenBank accession number
R55285, N35070, C00994 or AA221610 and provided that the
segment retains at least one biological activity of a
TNRL3 polypeptide.

20   In one embodiment, the invention provides a
soluble TNRL3 active fragment that includes a TNRL3
receptor binding domain. Such a soluble TNRL3 active
fragment can be, for example, a truncated polypeptide
encoding the extracellular domain of a TNRL3 polypeptide.
25 Soluble TNRL3 active fragments can have, for example, an
amino acid sequence that is identical or substantially
the same as amino acids 4 to 208 of hTNRL3 or amino acids
7 to 211 of mTNRL3, as shown in Figure 10. Such a
soluble TNRL3 active fragment lacks a functional
30 transmembrane domain. A soluble TNRL3 active fragment
also can be, for example, an active TNRL3 segment. Such
active TNRL3 segments are described hereinabove.

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As used herein, the term "substantially the
same amino acid sequence," when used in reference to a
polypeptide, fragment or segment of the invention, is
intended to mean a polypeptide, fragment or segment
5   having an identical amino acid sequence, or a
polypeptide, fragment or segment having a similar,
non-identical sequence that is considered by those
skilled in the art to be a functionally equivalent amino
acid sequence. For example, polypeptide including
10   substantially the same amino acid sequence as APO8 can
have an amino acid sequence identical to the sequence of
hAPO8 (SEQ ID NO:2) shown in Figure 2, or a similar,
non-identical sequence that is functionally equivalent.
An amino acid sequence that is "substantially the same"
15   can have one or more modifications such as amino acid
additions or substitutions relative to the amino acid
sequence shown, provided that the modified polypeptide
retains substantially at least one biological activity of
the polypeptide. Biological activities of the
20   polypeptides of the invention are described herein; for
example, biological activities of APO8 include the
ability to bind an APO8 ligand, bind the TRADD or RIP
adaptor molecules, induce or suppress apoptosis, activate
NF-κB expression, or induce or suppress cell
25   proliferation, differentiation or cytokine secretion.
Comparison of sequences for substantial similarity can be
performed between two sequences of any length and usually
is performed with nucleotide sequences of between 5 and
3500 nucleotides, preferably between about 10 and 300
30   nucleotides and more preferably between about 15 and 50
nucleotides. Comparison for substantial similarity
between amino acid sequences is usually performed with
sequences between about 6 and 1200 residues, preferably
between about 10 and 100 residues and more preferably
35   between about 25 and 35 residues. Such comparisons for

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substantial similarity are performed using methodology
routine in the art.

    Therefore, it is understood that limited
modifications can be made without destroying the
5 biological function of a polypeptide or ligand of the
invention. For example, minor modifications of hAPO8
(SEQ ID NO:2) that do not destroy polypeptide activity
also fall within the definition of APO8 and within the
definition of the polypeptide claimed as such.
10 Similarly, minor modifications of hAPO9 that do not
destroy polypeptide activity fall within the definition
of APO9; minor modifications of mAPO4-αL, mAPO4-αS,
hAPO4-α, rAPO4-α or mAPO4-β that do not destroy
polypeptide activity fall within the definition of an
15 APO4 polypeptide; and minor modifications of hAPO6 that
do not destroy polypeptide activity fall within the
definition of APO6. Similarly, minor modifications of
hTNRL1-α, mTNRL1-α, hTNRL1-β or mTNRL1-β that do not
destroy polypeptide activity fall within the definition
20 of TNRL1, and minor modifications of hTNRL3 and mTNRL3
that do not destroy polypeptide activity fall within the
definition of TNRL3. Also, for example, genetically
engineered fusion proteins that retain at least one
measurable biological activity of a polypeptide of the
25 invention fall within the definition of the polypeptides
claimed as such.

    It is understood that minor modifications of
primary amino acid sequence can result in polypeptides
which have substantially equivalent or enhanced function
30 as compared to the APO8 polypeptide sequence set forth in
Figure 2, substantially equivalent or enhanced function
as compared to the hAPO9 sequence set forth in Figure 6,
substantially equivalent or enhanced function as compared

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to the APO4 polypeptide sequences set forth in Figures 6,
7 and 8, or substantially equivalent or enhanced function
as compared to the hAPO6 sequence set forth in Figure 9.
Also, minor modifications of primary amino acid sequence
5   can result in polypeptides having substantially
equivalent or enhanced function as compared to the TNRL1
polypeptide sequences shown in Figures 11 and 12, or in
polypeptides having substantially equivalent or enhanced
function as compared to the TNRL3 polypeptide sequences
10   shown in Figure 13. These modifications can be
deliberate, as through site-directed mutagenesis, or can
be accidental such as through mutation in hosts harboring
an encoding nucleic acid. All such modified polypeptides
are included in the definition of an APO8 polypeptide as
15   long as at least one biological function of APO8 is
retained. Similarly, all such modified polypeptides are
included in the definition of an APO9, APO4, APO6, TNRL1
or TNRL3 polypeptide as long as at least one biological
function of an APO9, APO4, APO6, TNRL1 or TNRL3
20   polypeptide, respectively, is retained. Further, various
molecules can be attached to an APO8, APO9, APO4, APO6,
TNRL1 or TNRL3 polypeptide, for example, other
polypeptides, carbohydrates, lipids, or chemical
moieties. Such modifications are included within the
25   definition of each of the polypeptides of the invention.

Polypeptide fragments or segments to be
screened for activity can be produced, for example, by
recombinant methods or by chemical or proteolytic
cleavage of the isolated polypeptide. Methods for
30   chemical and proteolytic cleavage and for purification of
the resultant polypeptide fragments are well known in the
art as described above. (See, for example, Deutscher,
Methods in Enzymology, Vol. 182, "Guide to Protein

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Purification," San Diego: Academic Press, Inc. (1990),
which is incorporated herein by reference).

A polypeptide fragment or segment of the
invention can be screened, for example, for pro-apoptotic
5   or anti-apoptotic activity. Pro-apoptotic activity is
the ability either alone, or in combination with another
molecule, to produce cell death accompanied by at least
one of the morphological or biochemical alterations
characteristic of apoptosis. Morphological alterations
10 characteristic of apoptosis are well known in the art and
include, for example, condensed and rounded cellular
morphology; membrane blebbing; the formation of apoptotic
bodies, which are membrane-bound bodies containing
cytoplasmic and nuclear components; and condensation of
15 the nucleus, with cytoplasmic organelles being relatively
well maintained (Studzinski (Ed.), Cell Growth and
Apoptosis, Oxford: Oxford University Press (1995), which
is incorporated herein by reference). Biochemical
alterations characteristic of apoptosis also are well
20 known in the art. The classical biochemical alteration
characteristic of apoptosis is the appearance of
oligonucleosome-sized fragments of DNA, which produce a
"ladder" upon agarose gel electrophoresis. This
extensive fragmentation can be preceded by an earlier
25 endonucleolytic cleavage of chromatin, producing DNA
fragments of about 50 kb to 300 kb in size.

A variety of assays for determining whether a
polypeptide fragment or segment has pro-apoptotic or
anti-apoptotic activity are well known in the art. Such
30 methods include light microscopy for determining the
presence of one or more morphological characteristics of
apoptosis, such as condensed or rounded morphology,
shrinking and blebbing of the cytoplasm, preservation of

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structure of cellular organelles including mitochondria,
and condensation and margination of chromatin. As
described in Example II, cells expressing fragments of
hAPO8 were determined to be apoptotic by scoring for
5   condensed and rounded morphology.

      A polypeptide fragment or segment of the
invention also can be assayed for pro-apoptotic or
anti-apoptotic activity using terminal
deoxytransferase-mediated (TdT) dUTP biotin nick
10   end-labeling (TUNEL) (Gavriel et al., J. Cell Biol.
119:493 (1992), which is incorporated herein by
reference; Gorczyca et al., Int. J. Oncol. 1:639 (1992);
Studzinski, supra, 1995). ApopTag™ (ONCOR, Inc.,
Gaithersburg, MD) is a commercially available kit for
15   identification of apoptotic cells using digoxygenin
labeling. In addition, a polypeptide fragment or segment
of the invention can be assayed for pro-apoptotic or
anti-apoptotic activity by detecting nucleosomal DNA
fragments using agarose gel electrophoresis (Studzinski,
20   supra, 1995; Gong et al., Anal. Biochem. 218:314 (1994)).

      DNA filter elution methodology also can be used
to detect apoptosis-associated DNA fragmentation and to
determine pro-apoptotic or anti-apoptotic activity
(Studzinski, supra, 1995; Bertrand et al., Drug Devel.
25   34:138 (1995), which is incorporated herein by
reference). Pro-apoptotic or anti-apoptotic activity
also can be detected and quantitated by determining an
altered mitochondrial to nuclear DNA ratio as described
in Tepper et al., Anal. Biochem. 203:127 (1992) and
30   Tepper and Studzinski, J. Cell Biochem. 52:352 (1993),
each of which is incorporated herein by reference. One
skilled in the art understands that these, or other

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assays for pro-apoptotic or anti-apoptotic activity, can
be performed using methodology routine in the art.

        A nucleic acid to be assayed can encode a
polypeptide fragment or segment corresponding to a
5   portion of a native polypeptide of the invention or can
be modified to encode one or more amino acid
substitutions, deletions or insertions. One or more
point mutations can be introduced into the nucleic acid
encoding the modified polypeptide, fragment or segment to
10 be assayed using, for example, site-directed mutagenesis
(see Wu (Ed.), Meth. In Enzymol. Vol. 217, San Diego:
Academic Press (1993); Chapter 22 of Innis et al. (Ed.),
PCR Protocols, San Diego: Academic Press, Inc. (1990),
each of which is incorporated herein by reference). Such
15 mutagenesis can be used to introduce a specific, desired
amino acid substitution, deletion or insertion;
alternatively, a nucleic acid sequence can be synthesized
having random nucleotides at one or more predetermined
positions to generate random amino acid substitutions.
20 Scanning mutagenesis also can be useful in generating
nucleic acid molecules encoding, for example, an APO8
polypeptide, fragment or segment that is modified
throughout the entire sequence. Such a modified fragment
or segment can be screened, for example, for the ability
25 to bind an APO8 ligand; the ability to bind TRADD or RIP;
the ability to induce or suppress apoptosis or the
ability to activate NF-κB.

        A polypeptide of the invention can be obtained
by a variety of methods known within the art, including
30 biochemical, recombinant and chemical synthesis methods.
Biochemical methods for isolating a polypeptide of the
invention, or active fragment thereof, include
preparative gel electrophoresis, gel filtration, affinity

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chromatography, ion exchange and reversed phase
chromatography, chromatofocusing, isoelectric focusing
and sucrose or glycerol density gradients (see, for
example, Chapter 38 of Deutscher, Methods in Enzymology:
5   Guide to Protein Purification, Vol. 182, Academic Press,
Inc., San Diego (1990) and Chapter 8 of Balch et al.,
Methods in Enzymology, Vol. 257, Academic Press, Inc.,
San Diego (1995), each of which is incorporated herein by
reference in its entirety). For example, as disclosed
10 herein in Example I, human APO8 RNA is expressed in a
variety of human tissues including spleen, thymus,
prostate, testis, ovary, small intestine, colonic mucosa
and peripheral blood lymphocyte. From these results, one
skilled in the art knows that one of these tissues can be
15 used as a source of material for isolating an hAPO8
polypeptide of the invention.

Preparative gel electrophoresis can be useful
in preparing an isolated polypeptide, active fragment or
active segment of the invention. For example, an APO8
20 polypeptide, or an active fragment or segment thereof,
can be isolated by preparative polyacrylamide gel
electrophoresis and elution by diffusion or
electroelution (see, for example, Chapter 33 of
Deutscher, supra, 1990). Continuous elution gel
25 electrophoresis using a system such as the Model 491 Prep
Cell (BioRad, Hercules, CA) can be used to purify a
polypeptide or an active fragment or segment of the
invention. If desired, continuous elution gel
electrophoresis can be combined with further purification
30 steps such as liquid phase preparative isoelectric
focusing using, for example, the Rotofor system (BioRad).

Affinity chromatography is particularly useful
in preparing an isolated polypeptide or an active

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fragment or segment of the invention. For example, a
polypeptide that interacts with an APO8 polypeptide can
be useful as an affinity matrix for isolating an APO8
polypeptide or an active APO8 fragment or segment of the
5 invention. As disclosed herein, APO8 interacts
physically with TRADD and RIP (Example II), indicating
that TRADD or RIP can be used as an affinity matrix for
isolating an APO8 polypeptide. Death domain containing
polypeptides or fragments also can be useful as an
10 affinity matrix for isolating an APO8 polypeptide or an
active APO8 fragment or segment of the invention.

Immunoaffinity chromatography can be
particularly useful in isolating a polypeptide or an
active fragment or segment of the invention. For
15 example, immunoprecipitation or column chromatography
with an antibody that selectively binds APO8 can be used
to isolate an APO8 polypeptide or active fragment
thereof. An anti-APO8 monoclonal or polyclonal antibody
that selectively binds APO8 can be prepared using an
20 immunogen such as the sequence shown as SEQ ID NO:2, or a
synthetic peptide fragment thereof, as described further
below. Methods of affinity chromatography are well known
in the art and are described, for example, in Chapters
29, 30 and 38 of Deutscher, supra, 1990, which has been
25 incorporated herein by reference.

Recombinant methods for producing a polypeptide
through expression of a nucleic acid sequence in a
suitable host cell also are well known in the art and are
described, for example, in Sambrook et al., Molecular
30 Cloning: A Laboratory Manual, 2nd Ed, Vols 1 to 3, Cold
Spring Harbor Laboratory Press, New York (1989), which is
incorporated herein by reference. A nucleic acid for
expression of an APO8 polypeptide is provided herein as

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SEQ ID NO:1 and a nucleic acid for expression of an
APO8RP polypeptide is provided herein as SEQ ID NO:3. A
nucleic acid for expression of an APO9 polypeptide is
provided herein as SEQ ID NO:5; nucleic acids for
5 expression of APO4 polypeptides are provided herein as
SEQ ID NOS:7, 9, 11, 13 and 15; and a nucleic acid for
expression of an APO6 polypeptide is provided herein as
SEQ ID NO:17. Nucleic acids for expression of a TNRL1
polypeptide also are provided as SEQ ID NOS:19, 21, 23
10 and 25; and nucleic acids for expression of a TNRL3
polypeptide are provided herein as SEQ ID NOS:27 and 29.

       A recombinant polypeptide of the invention, or
active fragment or segment thereof, also can be expressed
as a fusion protein with a heterologous "tag" for
15 convenient isolation from bacterial or mammalian host
proteins. For example, histidine-tagged recombinant APO8
can be isolated by nickel-chelate chromatography.
Similarly, a glutathione-S-transferase tag or an
antigenic tag such as "FLAG," "AU" or a myc epitope tag
20 also can be included in a recombinant polypeptide or
active fragment of the invention (Sambrook et al., supra,
1989). The PinPoint™ expression system is a commercially
available system for expression of a polypeptide or
active fragment as a fusion protein with a heterologous
25 biotinylated peptide.

       A polypeptide or an active fragment or segment
of the invention also can be produced by chemical
synthesis, for example, by the solid phase peptide
synthesis method of Merrifield et al., J. Am. Chem. Soc.
30 85:2149 (1964), which is incorporated herein by
reference. Standard solution methods well known in the
art also can be used to synthesize a polypeptide or an
active fragment or segment useful in the invention (see,

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for example, Bodanszky, <u>Principles of Peptide Synthesis</u>,
Springer-Verlag, Berlin (1984) and Bodanszky, <u>Peptide
Chemistry</u>, Springer-Verlag, Berlin (1993), each of which
is incorporated herein by reference). A newly
5 synthesized polypeptide, fragment or segment can be
purified, for example, by high performance liquid
chromatography (HPLC) and can be characterized using mass
spectrometry or amino acid sequence analysis.

    Also provided herein are nucleic acid molecules
10 encoding the tumor necrosis family receptors and ligands
of the invention. These nucleic acid molecules are
useful, for example, in producing recombinant
polypeptides and as probes for diagnosing diseases
mediated by the polypeptides of the invention, as
15 described further below. A nucleic acid molecule of the
invention can have a nucleotide sequence of, for example,
about 15 to about 1500 nucleotides. In particular, a
nucleic acid molecule of the invention can have a
sequence of about 15, 18, 20, 25, 30, 35, 50, 100, 200,
20 500 or more nucleotides.

    The term "isolated," as used herein in
reference to a nucleic acid molecule of the invention,
means a nucleic acid molecule that is in a form that is
relatively free from contaminating lipids, polypeptides,
25 unrelated nucleic acids and other cellular material
normally associated with a nucleic acid molecule in a
cell.

    Provided herein is an isolated nucleic acid
molecule containing a nucleotide sequence encoding
30 substantially the same amino acid sequence as APO8, or an
active segment thereof, provided that the nucleic acid
molecule does not consist of the identical nucleotide

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sequence of an expressed sequence tag having GenBank
accession number AA223122 or AA232440. An isolated
nucleic acid molecule of the invention can have, for
example, a nucleotide sequence encoding substantially the
5   same amino acid sequence as hAPO8 (SEQ ID NO:2), or an
active segment thereof, provided that the nucleic acid
molecule does not consist of the identical nucleotide
sequence of an expressed sequence tag having GenBank
accession number AA223122 or AA232440. An exemplary
10   nucleic acid molecule of the invention encodes human APO8
and is provided herein as SEQ ID NO:1 (see Figure 2). In
one embodiment, the invention provides an isolated
nucleic acid molecule containing a nucleotide sequence
encoding a soluble APO8 active segment having an APO8
15   ligand binding domain.

          The invention further provides an isolated
nucleic acid molecule containing a nucleotide sequence
encoding substantially the same amino acid sequence as
APO9, or an active segment thereof, provided that the
20   nucleic acid molecule does not consist of the identical
nucleotide sequence of an expressed sequence tag having
GenBank accession number AA031883, AA150849, T71406 or
R10995. For example, the invention provides an isolated
nucleic acid molecule of the invention having a
25   nucleotide sequence encoding substantially the same amino
acid sequence as hAPO9 (SEQ ID NO:6), or an active
segment thereof, provided that the nucleic acid molecule
does not consist of the identical nucleotide sequence of
an expressed sequence tag having GenBank accession number
30   AA031883, AA150849, T71406 or R10995. An exemplary
nucleic acid molecule of the invention encodes human APO9
and is provided herein as SEQ ID NO:5 (see Figure 6). In
one embodiment, the invention provides an isolated
nucleic acid molecule containing a nucleotide sequence

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encoding a soluble APO9 active segment having an APO9 ligand binding domain.

In addition, the invention provides an isolated nucleic acid molecule containing a nucleotide sequence
5　encoding substantially the same amino acid sequence as an APO4 polypeptide, or an active segment thereof, provided that the nucleic acid molecule does not consist of the identical nucleotide sequence of an expressed sequence tag having GenBank accession number AA036247, AA003356,
10　W55289, AA445805 or W56629. Such a nucleic acid molecule can have a nucleotide sequence encoding substantially the same amino acid sequence as mAPO4-αL (SEQ ID NO:8), mAPO4-αS (SEQ ID NO:10), hAPO4-α (SEQ ID NO:12), rAPO4-α (SEQ ID NO:14), mAPO4-β (SEQ ID NO:16), or mAPO4-γ (SEQ
15　ID NO:32), or an active segment thereof, provided that the nucleic acid molecule does not consist of the identical nucleotide sequence of an expressed sequence tag having GenBank accession number AA036247, AA003356, W55289, AA445805 or W56629. Exemplary nucleic acid
20　molecules encoding the APO4 polypeptides of the invention are provided herein. As shown in Figure 7A-E, SEQ ID NO:7 is a nucleotide sequence encoding murine APO4-αL; SEQ ID NO:9 is a nucleotide sequence encoding murine APO4-αS; SEQ ID NO:11 is a nucleotide sequence encoding
25　human APO4-α; and SEQ ID NO:13 is a nucleotide sequence encoding rat APO4-α and SEQ ID NO:31 is a nucleotide sequence encoding murine APO4-γ. Figure 8 shows SEQ ID NO:15, which is a nucleotide sequence encoding murine APO4-β. The invention also provides an isolated nucleic
30　acid molecule containing a nucleotide sequence encoding a soluble active segment of an APO4 polypeptide having an APO4 ligand binding domain.

50

Also provided by the invention is an isolated
nucleic acid molecule containing a nucleotide sequence
encoding substantially the same amino acid sequence as
APO6, or an active segment thereof, provided that the
5   nucleic acid molecule does not consist of the identical
nucleotide sequence of an expressed sequence tag having
GenBank accession number AA025673, AA025672, AA155701,
AA155646, W67560 or AA158406. For example, the invention
provides an isolated nucleic acid molecule of the
10   invention having a nucleotide sequence encoding
substantially the same amino acid sequence as hAPO6 (SEQ
ID NO:18), or an active segment thereof, provided that
the nucleic acid molecule does not consist of the
identical nucleotide sequence of an expressed sequence
15   tag having GenBank accession number AA025673, AA025672,
AA155701, AA155646, W67560 or AA158406. Provided in
Figure 9 is an exemplary nucleic acid molecule of the
invention, SEQ ID NO:17, which encodes human APO6. In
one embodiment, the invention provides an isolated
20   nucleic acid molecule containing a nucleotide sequence
encoding a soluble APO6 active segment having an APO6
ligand binding domain.

The invention also provides an isolated nucleic
acid molecule containing a nucleotide sequence encoding
25   substantially the same amino acid sequence as a TNRL1
polypeptide, or an active segment thereof, provided that
the nucleic acid molecule does not consist of the
identical nucleotide sequence of an expressed sequence
tag having GenBank accession number AA166695, T87299,
30   R16882, AA254047, AA056924, AA057069, N91002, N79018 or
AA497494 or TIGR sequence number M78231. The invention
provides, for example, an isolated nucleic acid molecule
having a nucleotide sequence encoding substantially the
same amino acid sequence as hTNRL1-α (SEQ ID NO:20),

mTNRL1-α (SEQ ID NO:22), hTNRL1-β (SEQ ID NO:24) or
mTNRL1-β (SEQ ID NO:26) or an active segment thereof,
provided that the nucleic acid molecule does not consist
of the identical nucleotide sequence, or a segment

5   thereof, of an expressed sequence tag having GenBank
accession number AA166695, T87299, R16882, AA254047,
AA056924, AA057069, N91002, N79018 or AA497494 or TIGR
sequence number M78231. Provided in Figures 11 and 12
are exemplary nucleic acid molecules of the invention:

10 SEQ ID NO:19 encodes human TNRL1-α; SEQ ID NO:21 encodes
murine TNRL1-α; SEQ ID NO:23 encodes human TNRL1-β; and
SEQ ID NO:25 encodes murine TNRL1-β. The invention also
provides an isolated nucleic acid molecule containing a
nucleotide sequence encoding a soluble TNRL1 active

15 segment having a TNRL1 ligand binding domain.

        The invention further provides an isolated
nucleic acid molecule containing a nucleotide sequence
encoding substantially the same amino acid sequence as
TNRL3, or an active segment thereof, provided that the

20 nucleic acid molecule does not consist of the identical
nucleotide sequence of an expressed sequence tag having
GenBank accession number R55285, N35070, C00994 or
AA221610. For example, the invention provides an
isolated nucleic acid molecule of the invention having a

25 nucleotide sequence encoding substantially the same amino
acid sequence as hTNRL3 (SEQ ID NO:28) or mTNRL3 (SEQ ID
NO:30), or an active segment thereof, provided that the
nucleic acid molecule does not consist of the identical
nucleotide sequence of an expressed sequence tag having

30 GenBank accession number R55285, N35070, C00994 or
AA221610. Provided in Figure 13 are exemplary nucleic
acid molecules of the invention. SEQ ID NO:27 encodes
human TNRL3, and SEQ ID NO:29 encodes murine TNRL3. The
invention also provides an isolated nucleic acid molecule

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containing a nucleotide sequence encoding a soluble TNRL3
active segment having a TNRL3 ligand binding domain.

     The invention also provides selective binding
agents, which are agents that exhibit selective binding
5   for a receptor or ligand of the invention.

     Provided herein are APO8 selective binding
agents, which are agents that selectively bind an APO8
polypeptide or an active fragment thereof. Such an APO8
selective binding agent exhibits selective binding
10 affinity for an APO8 polypeptide, such as a polypeptide
having substantially the same amino acid sequence as
hAPO8 (SEQ ID NO:2) or active fragment thereof. In
addition to exhibiting selective binding affinity for
APO8, such APO8 selective binding agents can also
15 function to selectively enhance or inhibit the function
of APO8. For example, an APO8 selective binding agent
can function to enhance or inhibit binding of an APO8
polypeptide to its natural APO8 ligand, enhance or
inhibit binding to TRADD or RIP, enhance or inhibit
20 apoptotic activity, enhance or inhibit the capacity of
APO8 to activate NF-κB, or enhance or inhibit the ability
of an APO8 polypeptide to effect cell proliferation,
differentiation or cytokine secretion.

     The invention also provides APO9 selective
25 binding agents, which are agents that selectively bind an
APO9 polypeptide or active fragment thereof, such as a
polypeptide having, for example, substantially the same
amino acid sequence as hAPO9 (SEQ ID NO:6). Such APO9
selective binding agents exhibit selective binding
30 affinity for APO9 and, in addition, can function to
selectively enhance or inhibit the function of APO9. An
APO9 selective binding agent can, for example, enhance or

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inhibit APO9 binding to a ligand, enhance or inhibit the
pro-apoptotic or anti-apoptotic activity of an APO9
polypeptide, or enhance or inhibit the ability of an APO9
polypeptide to effect cell proliferation, differentiation
5 or cytokine secretion.

     Further provided herein are APO4 selective
binding agents, which are agents that selectively bind an
APO4 polypeptide or active fragment thereof. An APO4
selective binding agent can exhibit selective binding
10 affinity for an APO4 polypeptide having, for example,
substantially the same amino acid sequence as mAPO4-αL
(SEQ ID NO:8), mAPO4-αS (SEQ ID NO:10), hAPO4-α (SEQ ID
NO:12), rAPO4-α (SEQ ID NO:14), mAPO4-β (SEQ ID NO:16) or
mAPO4-γ (SEQ ID NO:32), or an active fragment thereof.
15 Such APO4 selective binding agents exhibit selective
binding affinity for an APO4 polypeptide and, in
addition, can selectively enhance or inhibit the function
of an APO4 polypeptide. An APO4 selective binding agent
can, for example, enhance or inhibit binding of an APO4
20 polypeptide to a ligand, enhance or inhibit pro-apoptotic
or anti-apoptotic activity of an APO4 polypeptide, or
enhance or inhibit the ability of an APO4 polypeptide to
effect cell proliferation, differentiation or cytokine
secretion.

25   APO6 selective binding agents also are provided
by the present invention. Such APO6 selective binding
agents are agents that selectively bind an APO6
polypeptide of the invention or active fragment thereof.
An APO6 selective binding agent can exhibit selective
30 binding affinity for an APO6 polypeptide having, for
example, substantially the same amino acid sequence as
hAPO6 (SEQ ID NO:18), or active fragment thereof. Such
APO6 selective binding agents exhibit selective binding

affinity for an APO6 polypeptide and, in addition, can selectively enhance or inhibit the function of an APO6 polypeptide. For example, an APO6 selective binding agent can enhance or inhibit the binding of an APO6

5   polypeptide to a ligand, enhance or inhibit the pro-apoptotic or anti-apoptotic activity of an APO6 polypeptide, or enhance or inhibit the ability of an APO6 polypeptide to effect cell proliferation, differentiation or cytokine secretion.

10   The invention additionally provides binding agents that selectively bind a ligand of the invention. Provided herein are TNRL1 selective binding agents, which are agents that selectively bind a TNRL1 polypeptide or active fragment thereof. A TNRL1 selective binding agent

15   can exhibit selective binding affinity for a TNRL1 polypeptide having, for example, substantially the same amino acid sequence as hTNRL1-α (SEQ ID NO:20), mTNRL1-α (SEQ ID NO:22), hTNRL1-β (SEQ ID NO:24) or mTNRL1-β (SEQ ID NO:26), or active fragments thereof. Such TNRL1

20   selective binding agents exhibit selective binding affinity for a TNRL1 polypeptide and, in addition, can selectively enhance or inhibit the function of a TNRL1 polypeptide. For example, a TNRL1 selective binding agent can enhance or inhibit the binding of a TNRL1

25   polypeptide to its cognate receptor.

            The invention further provides a TNRL3 selective binding agent, which is an agent that selectively binds a TNRL3 polypeptide or active fragment thereof. A TNRL3 selective binding agent can exhibit

30   selective binding affinity for a TNRL3 polypeptide having, for example, substantially the same amino acid sequence as hTNRL3 (SEQ ID NO:28) or mTNRL3 (SEQ ID NO:30), or active fragments thereof. Such TNRL3

selective binding agents exhibit selective binding
affinity for a TNRL3 polypeptide and, additionally, can
selectively enhance or inhibit the function of a TNRL3
polypeptide. For example, a TNRL3 selective binding
5   agent can enhance or inhibit the binding of a TNRL3
polypeptide to a cognate receptor.

          The term "selective binding agent," as used
herein, means an agent that exhibits selective binding to
the indicated polypeptide or polypeptide family. As used
10 herein, the term "agent" encompasses simple or complex
organic molecules, peptides, peptidomimetics,
polypeptides and antibodies as well as nucleic acids,
carbohydrates or lipids. A selective binding agent is
any such agent that binds with substantially higher
15 affinity to the indicated polypeptide or nucleic acid
than to an unrelated polypeptide or nucleic acid.

          Selective binding agents include natural
ligands as well as other agents that exhibit selective
binding for the indicated polypeptide of the invention.
20 Such agents include polyclonal or monoclonal antibodies
as well as selective binding agents isolated, for
example, from random bacteriophage libraries or
combinatorial libraries. Particularly useful selective
binding agents include activating antibodies that
25 selectively bind an APO8, APO9, APO4, APO6, TNRL1 or
TNRL3 polypeptide of the invention. Such activating
antibodies can, for example, function to cross-link
receptors and trigger receptor signaling in the absence
of ligand. Particularly useful selective binding agents
30 also include neutralizing antibodies, which can bind and
dampen or inactivate the function of an APO8, APO9, APO4,
APO6, TNRL1 or TNRL3 polypeptide. Particularly useful
selective binding agents that selectively bind a TNRL1 or

TNRL3 polypeptide of the invention also include soluble
or full-length cognate receptors, which bind their
respective TNRL1 or TNRL3 polypeptide ligand.

The term "selective binding," as used in
5   reference to a selective binding agent and an indicated
polypeptide, means that the agent binds with
substantially higher affinity to the indicated
polypeptide, or fragment thereof, than to another
polypeptide. For example, an APO8 selective binding
10 agent, which selectively binds an APO8 polypeptide, binds
with substantially higher affinity to an APO8
polypeptide, or fragment thereof, than to another member
of the TNFR superfamily such as TNFR-1 or another death
domain containing polypeptide such as FADD. Thus, for
15 example, an agent that binds the death domains of TNFR-1,
FAS/APO-1 and APO8 with similar affinity is not a
selective binding agent as defined herein. However, an
agent that has substantially higher affinity for an APO8
death domain than for the TNFR-1 and FAS/APO-1 death
20 domains exhibits selective binding for APO8 and,
therefore, is encompassed within the definition of an
APO8 selective binding agent. Similarly, a TNRL1
selective binding agent, which as defined herein is an
agent that selectively binds a TNRL1 polypeptide, binds
25 with substantially higher affinity to a TNRL1 polypeptide
than to another member of the TNF family such as TNF-α.

The invention also provides a method of
identifying an APO8 selective binding agent by contacting
an APO8 polypeptide, or fragment thereof, with an agent
30 and determining selective binding of the agent to the
APO8 polypeptide, where said the selective binding
indicates that the agent is an APO8 selective binding
agent.

A variety of methods for determining the
selective binding of an agent to a polypeptide or
fragment of the invention are well known in the art.
Such methods include, for example, ELISA, RIA,
5 receptor/ligand binding assays and other affinity assays
such as chromatographic methods and panning. These
screening methods are well known in the art and are
described, for example in Harlow et al., Antibodies: A
Laboratory Manual, Cold Spring Harbor Laboratory, New
10 York (1992); Ansubel et al., Current Protocols in
Molecular Biology, John Wiley and Sons, Baltimore, MD
(1989) and in Devlin, T.M., Textbook of Biochemistry with
Clinical Correlations, Wiley-Liss, New York, NY (1992).

Screening assays for detecting selective
15 binding also include two-hybrid systems such as the yeast
two hybrid system, which can be used to screen a panel of
agents to detect selective binding to a polypeptide of
the invention or fragment thereof (see Fields and Song,
Nature 340:245-246 (1989), which is incorporated herein
20 by reference) or a two-hybrid system adapted for use in
mammalian cells (see Fearon et al., Proc. Natl. Acad.
Sci., USA 89:7958-7962 (1992), which is incorporated
herein by reference).

Using one of the assays described above, or
25 another assay well known in the art, a large collection,
or library, of random agents or agents or interest can be
screened for selective binding activity. Polypeptide
libraries and tagged chemical libraries including those
comprising polypeptides, peptides and peptidomimetic
30 molecules can be screened. Polypeptide libraries also
include those generated by phage display technology.
Phage display technology includes the expression of
polypeptide molecules on the surface of phage as well as

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other methodologies by which a protein ligand is or can
be associated with the nucleic acid which encodes it.
Methods for production of phage display libraries,
including vectors and methods of diversifying the
5   population of peptides which are expressed, are well
known in the art (see, for example, Smith and Scott,
Methods Enzymol. 217:228-257 (1993); Scott and Smith,
Science 249:386-390 (1990); and Huse, WO 91/07141 and WO
91/07149, each of which is incorporated herein by
10   reference). These or other well known methods can be
used to produce a phage display library which can be
screened, for example, to identify a selective binding
agent of the invention.

       Each of the isolated receptor polypeptides
15   provided herein are useful as immunogens for preparing an
antibodies. Thus, the invention provides an antibody
that selectively binds an APO8 polypeptide, for example,
hAPO8 (SEQ ID NO:2). Such an antibody is useful, for
example, in purifying an APO8 polypeptide by
20   immunoaffinity chromatography.

       The invention further provides an antibody that
selectively binds an APO9 polypeptide of the invention,
for example, hAPO9 (SEQ ID NO:6). Similarly, the
invention provides an antibody that selectively binds an
25   APO4 polypeptide; such an antibody can selectively bind,
for example, mAPO4-αL (SEQ ID NO:8), mAPO4-αS (SEQ ID
NO:10), hAPO4-α(SEQ ID NO:12), rAPO4-α (SEQ ID NO:14),
mAPO4-β (SEQ ID NO:16) or mAPO4-γ (SEQ ID NO:32).
Further provided by the invention is an antibody that
30   selectively binds an APO6 polypeptide, for example, hAPO6
(SEQ ID NO:18). Each of these antibodies are useful in
purifying the polypeptide to which it selectively binds
using immunoaffinity chromatography.

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The isolated ligand polypeptides provided
herein also are useful as immunogens for preparing
antibodies. Thus, the invention provides antibodies that
selectively bind a ligand of the invention. Provided
5   herein is an antibody that selectively binds a TNRL1
polypeptide, for example, hTNRL1-α (SEQ ID NO:20),
mTNRL1-α (SEQ ID NO:22), hTNRL1-β (SEQ ID NO:24) or
mTNRL1-β (SEQ ID NO:26) or an active fragment thereof.
Also provided herein is an antibody that selectively
10 binds a TNRL3 polypeptide of the invention. Such an
antibody can selectively bind, for example, hTNRL3 (SEQ
ID NO:28) or mTNRL3 (SEQ ID NO:30).

As used herein, the term "antibody" is used in
its broadest sense to include polyclonal and monoclonal
15 antibodies, as well as polypeptide fragments of
antibodies that retain a selective binding activity for a
polypeptide of the invention of at least about 1×10^5
M^{-1}. One skilled in the art would know that antibody
fragments such as Fab, $F(ab')_2$ and Fv fragments can retain
20 selective binding activity for a polypeptide of the
invention and, thus, are included within the definition
of an antibody. In addition, the term antibody as used
herein includes naturally occurring antibodies, as well
as non-naturally occurring antibodies and fragments such
25 as chimeric antibodies and humanized antibodies that have
selective binding activity. Such non-naturally occurring
antibodies can be constructed using solid phase peptide
synthesis, produced recombinantly or obtained, for
example, by screening combinatorial libraries consisting
30 of variable heavy chains and variable light chains as
described by Borrebaeck (Ed.), Antibody Engineering
(Second edition) New York: Oxford University Press
(1995), which is incorporated herein by reference.

Methods for producing antibodies are routine in the art. A purified polypeptide of the invention, which can be prepared from natural sources or produced recombinantly as described above, or a fragment thereof,
5 such as a synthetic peptide, can be used as an immunogen. Non-immunogenic fragments or synthetic peptides can be made immunogenic by coupling the hapten to a carrier molecule such as bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH). In addition, various other
10 carrier molecules and methods for coupling a hapten to a carrier molecule are well known in the art as described, for example, by Harlow and Lane, Antibodies: A Laboratory Manual (Cold Spring Harbor Laboratory Press, 1988), which is incorporated herein by reference.

15   An antibody "having selective binding for" a polypeptide, or that "selectively binds" a polypeptide, binds with substantially higher affinity to that polypeptide than to another polypeptide.

        The APO8 encoding nucleic acids and
20 polypeptides of the invention can be used to diagnose, treat or reduce the severity of an APO8-mediated disease. For example, the APO8 encoding nucleic acids, polypeptides and active fragments and segments thereof can be used for diagnosis of an APO8-mediated disease or
25 can be used to generate reagents useful for such diagnosis. Diagnosis can be performed, for example, by nucleic acid probe hybridization, or by amplification of APO8 encoding nucleotide sequences and subsequent electrophoretic analysis such as DNA sequencing.
30 Diagnosis also can be performed, for example, using antibody or ligand based detection with an APO8 selective binding agent. Detection can be performed *ex vivo*, for example, by removing a cell or tissue sample from an

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individual at risk of, suspected of having or exhibiting
one or more symptoms of an APO8-mediated disease.
Altered APO8 expression or activity is indicative of an
APO8-mediated disease. As used herein, the term
5   "APO8-mediated disease" means a disease, pathology, or
other condition resulting from an abnormality in the
expression or activity of an APO8 polypeptide. Included
within the meaning of the term "APO8-mediated disease"
are disorders of cell loss, disorders of cell
10 accumulation and disorders of differentiation, as
described further below.

          The present invention provides a method of
treating or reducing the severity of an APO8-mediated
disease in a subject by administering an APO8 polypeptide
15 or active fragment thereof or by administering a nucleic
acid molecule encoding the APO8 polypeptide or fragment.
An APO8 polypeptide or nucleic acid molecule useful in
such a method can be or encode, for example, an amino
acid sequence that is identical or substantially the same
20 as hAPO8 (SEQ ID NO:2), or an active fragment thereof.
An APO8 antisense nucleic acid molecule, which has a
nucleotide sequence that is identically or substantially
complementary to an APO8 encoding nucleic acid or active
fragment thereof, also can be used to treat or reduce the
25 severity of an APO8-mediated disease according to a
method of the invention.

          Also provided herein is a method of treating or
reducing the severity of an APO8-mediated disease in a
subject by administering an APO8 regulatory agent to the
30 subject. As used herein, an "APO8 regulatory agent" is
an agent that inhibits or enhances a biological activity
of an APO8 polypeptide. Such an APO8 regulatory agent
can be, for example, an APO8 inhibitory agent such as a

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dominant negative form of APO8, an APO8 selective binding
agent that inhibits a biological activity of an APO8
polypeptide, or a cysteine protease inhibitor. Dominant
negative forms of APO8 include, for example, soluble
5   forms of APO8 having an APO8 ligand binding domain, as
well as membrane bound forms of APO8 having an APO8
ligand binding domain but lacking a functional
cytoplasmic tail, known as "decoy receptors." Examples
of such "decoy receptor" APO8 inhibitory agents are
10 provided herein as APO8ΔCP and APO8ΔDD (see Example II).

An APO8 regulatory agent also can be an agent
that inhibits or enhances the activity or expression of
FADD, FLICE, TRADD or RIP or that inhibits or enhances
the activity or expression of NF-κB. One skilled in the
15 art understands that such an APO8 regulatory agent can be
an agent that selectively regulates a biological activity
of an APO8 polypeptide or, alternatively, can be a
non-selective agent that, in addition to regulating a
biological activity of an APO8 polypeptide, also
20 regulates the activity of, for example, other death
domain containing receptors. As shown in Example II, the
FADD dominant negative construct DN-FADD, the FLICE
dominant negative construct (FL-C360S) and the cysteine
protease inhibitors CrmA and z-VAD-fmk each inhibit APO8
25 induced apoptosis. In addition, the TRADD dominant
negative construct ND-TRADD inhibits APO8-mediated NF-κB
activation, which can protect cells from apoptosis
(Example II). Thus, each of these molecules are examples
of APO8 regulatory agents as defined herein.

30   As described above, an agent can be a simple or
complex organic molecule, peptide, peptidomimetic,
polypeptide, antibody, nucleic acid, carbohydrate or
lipid molecule.

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The present invention also provides a method of
treating or reducing the severity of an APO8RP-mediated
disease in a subject by administering to the subject a
FADD regulatory agent. Such a FADD regulatory agent is
5 an agent, such as a simple or complex organic molecule,
peptide, peptidomimetic, polypeptide, antibody, nucleic
acid, carbohydrate or lipid molecule, that inhibits or
enhances FADD activity or expression. As disclosed
herein, hAPO8 (SEQ ID NO:2) and hAPO8RP (SEQ ID NO:4)
10 induce apoptosis through the FADD adaptor molecule. The
data shown in Figure 5B demonstrate that a FADD dominant
negative construct (DN-FD) reduces APO8RP-mediated
apoptosis. These results indicate that a FADD regulatory
agent, such as a FADD dominant negative construct, can be
15 used to reduce APO8RP-induced apoptosis and, thus, treat
or reduce the severity of an APO8RP-mediated disease. An
"APO8 related polypeptide-mediated disease," as used
herein, is synonymous with "APO8RP-mediated disease" and
means a disease, pathology, or other condition resulting
20 from an abnormality in the expression or activity of an
APO8RP polypeptide and can be, for example, a disorder of
cell loss, disorder of cell accumulation or disorder of
cell differentiation. An APO8RP-mediated disease also
can be, for example, an autoimmune disorder.

25   Further provided by the present invention is a
method of treating or reducing the severity of an
APO8RP-mediated disease in a subject by administering to
the subject a TRADD regulatory agent or a RIP regulatory
agent. Such an agent enhances or inhibits the activity
30 or expression of TRADD or RIP. As disclosed herein in
Example II, both APO8 and APO8RP directly interact with
the TRADD and RIP adaptor molecules. Example II further
demonstrates that a TRADD dominant negative construct
blocks NF-κB activation by APO8 and APO8RP. Thus, a

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TRADD regulatory agent or a RIP regulatory agent, such as
a TRADD dominant negative construct, can be used to treat
or reduce the severity of an APO8RP-mediated disease.

5   The present invention also provides a method of
treating or reducing the severity of an APO8RP-mediated
disease in a subject by administering to the subject an
NF-κB regulatory agent, which is an agent that enhances
or inhibits the activity or expression of NF-κB.
Activation of the NF-κB pathway is known to protect cells
10   from apoptosis, and, as disclosed herein, expression of
APO8 or APO8RP results in NF-κB activation (see
Example II). Thus, an NF-κB regulatory agent can block,
for example, apoptosis and, therefore, can be useful in
treating or reducing the severity of an APO8RP-mediated
15   disease that results from excessive cell death.

       The APO9 encoding nucleic acids and
polypeptides of the invention can be used to diagnose,
treat or reduce the severity of an APO9-mediated disease.
For example, the APO9 encoding nucleic acids,
20   polypeptides and active fragments thereof can be used for
diagnosis of an APO9-mediated disease or can be used to
generate reagents useful for such diagnosis. Diagnosis
can be performed, for example, by nucleic acid probe
hybridization, or by amplification of APO9 encoding
25   nucleotide sequences and subsequent electrophoretic
analysis such as DNA sequencing. Diagnosis also can be
performed using, for example, antibody or ligand based
detection with an APO9 selective binding agent.
Detection can be performed ex vivo, for example, by
30   removing a cell or tissue sample from an individual at
risk of, suspected of having or exhibiting one or more
symptoms of an APO8-mediated disease. Altered APO9
expression or activity is indicative of an APO9-mediated

disease. As used herein, the term "APO9-mediated
disease" means a disease, pathology, or other condition
resulting from an abnormality in the expression or
activity of an APO9 polypeptide. Included within the
5   meaning of the term "APO9-mediated disease" are disorders
of cell loss, disorders of cell accumulation and
disorders of cell differentiation.

        The present invention provides a method of
    treating or reducing the severity of an APO9-mediated
10 disease in a subject by administering an APO9 polypeptide
    or active fragment thereof or by administering a nucleic
    acid molecule encoding the APO9 polypeptide or fragment.
    An APO9 polypeptide or nucleic acid molecule useful in
    such a method can be or encode, for example, an amino
15 acid sequence identical or substantially the same as
    hAPO9 (SEQ ID NO:6), or an active fragment thereof. An
    APO9 antisense nucleic acid molecule, which has a
    nucleotide sequence that is identically or substantially
    complementary to an APO9 encoding nucleic acid or active
20 fragment thereof, also can be used to treat or reduce the
    severity of an APO9-mediated disease according to a
    method of the invention.

        Further provided herein is a method of treating
    or reducing the severity of an APO9-mediated disease in a
25 subject by administering an APO9 regulatory agent to the
    subject. As used herein, an "APO9 regulatory agent" is
    an agent that inhibits or enhances a biological activity
    of an APO9 polypeptide. Such an APO9 regulatory agent
    can be, for example, an APO9 inhibitory agent such as a
30 dominant negative form of APO9, an alternative spliced
    full-length form of APO9, or an APO9 selective binding
    agent that inhibits a biological activity of an APO9
    polypeptide. Dominant negative forms of APO9 include,

for example, soluble forms of APO9 having an APO9 ligand
binding domain, as well as membrane bound forms of APO9
having an APO9 ligand binding domain but lacking a
functional cytoplasmic tail, known as "decoy receptors."
5   An APO9 regulatory agent can also be an agent that
selectively binds an alternative spliced full-length form
of APO9, thereby blocking or transmitting a signal
through full-length APO9. One skilled in the art
understands that such an APO9 regulatory agent can be an
10 agent that selectively regulates a biological activity of
an APO9 polypeptide or, alternatively, can be a
non-selective agent that, in addition to regulating a
biological activity of an APO9 polypeptide, also
regulates the activity of, for example, other TNF family
15 receptors.

        The APO4 encoding nucleic acids and
polypeptides of the invention can be used to diagnose,
treat or reduce the severity of an APO4-mediated disease.
The APO4 encoding nucleic acids, polypeptides and active
20 fragments thereof can be used for diagnosis of an
APO4-mediated disease or can be used to generate reagents
useful for such diagnosis. Diagnosis can be performed,
for example, by nucleic acid probe hybridization, or by
amplification of APO4 encoding nucleotide sequences and
25 subsequent electrophoretic analysis such as DNA
sequencing. Diagnosis also can be performed, for
example, using antibody or ligand based detection with an
APO4 selective binding agent. Detection can be performed
ex vivo, for example, by removing a cell or tissue sample
30 from an individual at risk of, suspected of having or
exhibiting one or more symptoms of an APO4-mediated
disease. Altered APO4 polypeptide expression or activity
can be indicative of an APO4-mediated disease. As used
herein, the term "APO4-mediated disease" means a disease,

pathology, or other condition resulting from an
abnormality in the expression or activity of an APO4
polypeptide. Included within the meaning of the term
"APO4-mediated disease" are disorders of cell loss,
5   disorders of cell accumulation and disorders of cell
differentiation.

        As disclosed herein, APO4 was expressed highly
in embryonic tissue at day 11, 15 and 17, indicating that
APO4 can play a role in early development (see
10   Example IV). APO4 expression in embryonic tissues also
indicates that APO4 can play a role in developmental
abnormalities or in gestation. Thus, APO4 and can be
used in the diagnosis or treatment of developmental or
gestational abnormalities.

15   The present invention provides, for example, a
method a diagnosing a developmental abnormality caused by
the aberrant expression of APO4 during development by
determining the level or pattern of expression of APO4
mRNA or protein in embryonic cells or tissue. The
20   invention further provides a method of diagnosing a
developmental abnormality casued by the aberrant
expression of APO4 during development by detecting a
mutation in an APO4 gene in a cell or tissue derived from
embryos.

25   In adult tissues, APO4 expression was highest
in adult prostate, with lower levels of expression seen
in adult spleen, thymus, testis, uterus, small intestine,
colon and peripheral blood leukocytes. APO4 also was
highly expressed in a prostate carcinoma epithelial cell
30   line, LNCaP (see Example IV). Since the glandular
epithelial cells of the prostate represent the site of
origin of prostate cancer and the cell type that

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increases during prostate cancer growth, increased APO4
levels can be used as a marker diagnostic of the presence
of prostate cancer. For example, as the levels of APO4
secreted by prostatic tissue increase with the increasing
5   mass of the prostate epithelial cells, elevated levels of
APO4 either in the serum or from a tissue biopsy of an
individual suspected of having prostate cancer can be
used to diagnose the existence of prostate cancer. In
addition, altered APO4 levels can be assayed in an
10   individual with prostate cancer as a method of staging
the degree or severity of the cancer, thereby providing
critical information for determining the most
advantageous therapeutic strategy. Altered APO4 levels
also can be useful for the diagnosis of local and distant
15   metastatic spread of prostate cancer and for assessment
of disease volume. Such a diagnostic indicator is
particularly useful in planning radiation therapy for
prostate cancer by defining the limits of the spread of
disease.

20   Thus, the present invention provides a method
of diagnosing prostate cancer in an individual by
determining the level of APO4 in the individual. A
method of the invention can be particularly useful in
diagnosing prostate cancer in an individual at risk for
·25   this cancer, such as in men over the age of fifty or with
a family history of prostate cancer.

           Soluble APO4 can be a naturally occurring form
secreted into the bloodstream. For example, murine APO4
form occurs naturally in soluble form and is secreted
30   into the bloodstream. In one embodiment, the invention
provides a method of diagnosing prostate cancer in an
individual by determining the level of soluble APO4 in
the individual. Such methods can be particularly useful

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since a non-invasive blood test can be used in order to
determine the level of soluble APO4.

        A variety of methods can be used to assay for
the level of APO4 according to a method of the invention
5 for diagnosing prostate cancer.   Such methods include
assays for APO4 RNA levels, for example, reverse
transcriptase polymerase chain reaction (RT-PCR)
analysis.   Such methods further include immunoassays for
determining the level of APO4 polypeptide, for example,
10 radioimmunoassays and enzyme-linked immunoassays.   The
level of APO4 in an individual suspected of having
prostate cancer can be determined, for example, in a
sample such as a serum sample from the individual.   If
desired, the level of APO4 also can be assayed in situ,
15 for example, by administering an anti-APO4 antibody
linked to a detectable moiety and subsequent detection of
the moiety.

        The invention also provides a method of
identifying an effective pharmacological agent useful in
20 the diagnosis or treatment of a disease associated with
APO4 activity.   The method includes the steps of
contacting an APO4 polypeptide or active
fragment thereof with an agent; and determining selective
binding of the agent to the APO4 polypeptide or
25 active fragment thereof, where the selective binding
indicates that the agent is an effective pharmacological
agent useful in the diagnosis or treatment of a disease
associated with APO4 activity.   An APO4 polypeptide or
active fragment thereof useful in the invention can be,
30 for example, an APO4 extracellular ligand binding domain.
In one embodiment, the APO4 polypeptide or active
fragment thereof is expressed in a cell or on the surface
of a cell.   In another embodiment, the APO4 polypeptide

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or active fragment thereof is contacted with an agent *in vitro*.

Methods for determining selective binding are known in the art and are described hereinabove. For
5   example; assays to determine selective binding include, two-hybrid systems, ELISA, RIA, receptor/ligand binding assays and other affinity assays such as chromatographic methods and panning.

The present invention also provides a method of
10 screening for an APO4 agonist useful in treating prostate cancer. The method includes the steps of contacting a cell expressing an APO4 polypeptide or active fragment thereof with an agent; and assaying for increased APO4 activity, where increased APO4 activity indicates that
15 the agent is an APO4 agonist useful in treating prostate cancer.

An APO4 activity can be, for example, APO4 binding to a ligand, activation of the JNK pathway or activation of NF-κB activity. APO4 activity also
20 includes apoptotic activity in a cell in response to increased APO4 expression or contact with APO4. The stimulation or induction of apoptosis in the cell includes any increased level of apoptosis compared to the level observed prior to contact with the APO4 agonist.

25 Further provided herein is a method of screening for an APO4 antagonist. The method includes the steps of contacting a cell expressing an APO4 polypeptide or active fragment thereof with an agent; and assaying for decreased APO4 activity, where decreased
30 APO4 activity indicates that the agent is an APO4 antagonist.

The invention also provides a method of
identifying an effective pharmacological agent useful in
the diagnosis or treatment of a disease associated with
APO4 activity by forming a mixture including an APO4
5   polypeptide or active fragment thereof, an APO4 signal
transducer molecule that specifically interacts with a
cytoplasmic domain of the APO4 polypeptide or active
fragment thereof, and an agent; and detecting the level
of APO4 activity in the presence of the agent, where an
10 alteration in the APO4 activity relative to control
activity indicates that the agent is an effective
pharmacological agent useful in the diagnosis or
treatment of a disease associated with APO4 activity. An
alteration in APO4 activity can be an increased or
15 decreased activity. Examples of APO4 activity include,
for example, activation of the JNK pathway, activation of
NF-κB, apoptosis, cell proliferation or cell
differentiation. In such a method of the invention, the
APO4 activity can be measured, for example, *in vivo* or *in*
20 *vitro*.

As discussed above, APO4 can be highly
expressed in prostate cancer epithelial cells. Based on
this expression, the present invention also provides a
method of treating prostate cancer by administering to an
25 individual having prostate cancer a conjugate including
an APO4 selective binding agent linked to a therapeutic
moiety. Administration of a conjugate including an APO4
selective binding agent, for example, an anti-APO4
monoclonal antibody, facilitates targeted delivery of a
30 linked therapeutic moiety such as doxorubicin to prostate
cancer epithelial cells.

As used herein, the term "APO4 selective
binding agent" means a simple or complex organic

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molecule, peptide, peptidomimetic, polypeptide, antibody,
nucleic acid, carbohydrate or lipid that binds with
substantially higher affinity to an APO4 polypeptide than
to an unrelated polypeptide. Methods for identifying a
5   selective binding agent are described hereinabove.

As disclosed herein, a therapeutic moiety can
be, for example, a cancer chemotherapeutic agent linked
to an APO4 selective binding agent to produce a conjugate
containing an APO4 selective binding agent linked to a
10 therapeutic moiety. Cytotoxic chemotherapy is the basis
of the systemic treatment of disseminated malignant
tumors. However, a major limitation of the currently
used chemotherapeutic agents is that these drugs have the
narrowest therapeutic index in all of medicine. As such,
15 the dose of cancer chemotherapeutic agents generally is
limited by undesirable toxicity to the patient being
treated. Thus, the ability of an APO4 selective binding
agent to target a drug to cancerous prostate tissue
allows for a higher amount of chemotherapeutic agent to
20 be delivered directly to the cancer with reduced side
effects.

As used herein, the term "therapeutic moiety"
means a physical, chemical, or biological material that
is linked to an APO4 selective binding agent for the
25 purpose of being targeted in vivo to an APO4 polypeptide.
A therapeutic moiety can be a cancer chemotherapeutic
agent such as doxorubicin, which, when linked to an APO4
selective binding agent, provides a conjugate useful for
treating a cancer in a subject. In addition, a
30 therapeutic moiety can be a drug delivery vehicle such as
a chambered microdevice, a cell, a liposome or a virus,
which can contain an agent such as a drug or a nucleic
acid. An APO4 selective binding agent also can be linked

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to a therapeutic moiety expressed by a virus, for
example, the adenovirus penton base coat protein, thus
providing a means to target a virus to cancerous prostate
cells (Wickman et al., Gene Ther. 2:750-756 (1995);
5   Weitzman et al., In: "Gene Therapy and Vector Systems"
2:17-25 (1997), each of which is incorporated herein by
reference).

        A therapeutic moiety also can be, for example,
a radioactive moiety or can be a cytotoxic agent,
10 including a toxin such as ricin or a drug such as a
chemotherapeutic agent or can be a physical, chemical or
biological material such as a liposome, microcapsule,
micropump or other chambered microdevice, which can be
used, for example, as a drug delivery system. Generally,
15 such microdevices, should be nontoxic and, if desired,
biodegradable. Various moieties including microcapsules,
which can contain an agent, are well known in the art and
commercially available (see, for example, "Remington's
Pharmaceutical Sciences" 18th ed. (Mack Publishing Co.
20 1990), chapters 89-91; Harlow and Lane, Antibodies: A
laboratory manual (Cold Spring Harbor Laboratory Press
1988), each of which is incorporated herein by
reference).

25   In one embodiment, an APO4 selective binding
agent for treating prostate cancer is a monoclonal or
polyclonal antibody. Use of antibody-conjugated
immunotoxins and radioactive moieties for the treatment
of cancer are well known in the art, as described, for
30 example, by Geissler et al. Cancer Res., 52:2907-2915
(1992); Thrush et al., Ann Rev. Immunol., 14:49-71
(1996); and Trail et al., Science, 261:212-215 (1993).
For example, the anthracyclin, doxorubicin, has been
linked to antibodies and the antibody/doxorubicin

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conjugated have been therapeutically effective in
treating tumors (Sivan et al., Cancer Res. 55:2352-2356
(1995); Lau et al., Bioorg. Med. Chem. 3:1299-1304
(1995); Shih et al., Cancer Immunol. Immunother. 38:92-98
5   (1994), which are incorporated herein by reference).
Similarly, other anthracyclins, including idarubicin and
daunorubicin, have been chemically conjugated to
antibodies, which have delivered effective doses of the
therapeutic moiety to tumors (Rowland et al., Cancer
10   Immunol. Immunother. 37:195-202 (1993); Aboud-Pirak et
al., Biochem. Pharmacol. 38:641-648 (1989)). Thus, an
anthracyclin, such as doxorubicin, idarubicin or
daunorubicin, is a therapeutic moiety particularly useful
in the methods of the invention.

15   A therapeutic moiety can be conjugated to a
selective binding agent using a variety of methods. A
covalent bond can be formed, for example, using
glutaraldehyde, a heterobifunctional cross-linker or a
homobifunctional cross-linker. Additional conjugation
20   methods include carbodiimide conjugation methods
(Bauminger and Wilchek, Meth. Enzymol. 70:151-159 (1980),
which is incorporated herein by reference).
Carbodiimides comprise a group of compounds that have the
general formula R-N=C=N-R', where R and R' can be
25   aliphatic or aromatic, and are used for synthesis of
peptide bonds. The preparative procedure is simple,
relatively fast, and is carried out under mild
conditions. Carbodiimide compounds attack carboxylic
groups to change them into reactive sites for free amino
30   groups. Carbodiimide conjugation has been used to
conjugate a variety of compounds to carriers for the
production of antibodies.

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In addition to using carbodiimides for
conjugation, the water soluble carbodiimide, 1-ethyl-3-
(3-dimethylaminopropyl) carbodiimide (EDC) also can be
used for conjugating a therapeutic moiety to an APO4
5   selective binding agent. EDC can also be used to prepare
active esters such as N-hydroxysuccinimide (NHS) ester.
The NHS ester, which binds only to amino groups, then can
be used to induce the formation of an amide bond with the
single amino group of the doxorubicin. The use of EDC
10 and NHS in combination is commonly used for conjugation
in order to increase the yield of conjugate formation
(Bauminger and Wichek, supra, 1980).

Other methods for conjugating a therapeutic
moiety to an APO4 selective binding agent also can be
15 used. For example, sodium periodate oxidation followed
by reductive alkylation of appropriate reactants can be
used, as can glutaraldehyde cross-linking. The
cross-linking of proteins can additionally be
accomplished by using reactive groups within the
20 individual protein such as carbohydrate, disulfide,
carboxyl or amino groups. Coupling can also be
accomplished by oxidation or reduction of the native
protein, or treatment with an enzyme, for example.
However, it is recognized that, regardless of which
25 method of producing a conjugate is selected, a
determination must be made that the APO4 selective
binding agent maintains its targeting ability and that
the therapeutic moiety maintains its relevant function.

The yield of therapeutic moiety/selective
30 binding agent conjugate formed is determined using
routine methods. For example, HPLC or capillary
electrophoresis or other qualitative or quantitative
method can be used (see, for example, Liu et al., J.

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Chromatogr. 735:357-366 (1996); Rose et al., _J._
Chromatogr. 425:419-412 (1988), each of which is
incorporated herein by reference). In particular, the
skilled artisan will recognize that the choice of a
5   method for determining yield of a conjugation reaction
depends, in part, on the physical and chemical
characteristics of the specific therapeutic moiety and
selective binding agent. Following conjugation, the
reaction products are desalted to remove any free peptide
10 and free drug.

A conjugate made up of an APO4 selective
binding agent linked to a therapeutic moiety can be
administered to an individual having prostate cancer, or,
if desired, to an individual suspected of having prostate
15 cancer or an individual at risk of developing prostate
cancer. Administration can be accomplished, for example,
by intravenous, intraperitoneal or subcutaneous
injection. A conjugate made up of an APO4 selective
binding agent linked to a therapeutic moiety can be
20 administered by conventional methods using a dosage
sufficient to reduce the growth or severity of the
prostate cancer. Such dosages can be readily determined
by those skilled in the art and include a variety of
different regimes, including single high dose
25 administration or repeated small dose administration or a
combination of both. The dosing will depend on the
progression of the disease and overall health of the
individual and can be determined by those skilled in the
art.

30   The present invention also provides a method of
treating or reducing the severity of an APO4-mediated
disease in a subject by administering an APO4 polypeptide
or active fragment thereof or by administering a nucleic
acid molecule encoding the APO4 polypeptide or fragment.

An APO4 polypeptide or nucleic acid molecule useful in
such a method can be or encode, for example, an amino
acid sequence identical or substantially the same as
mAPO4-αL (SEQ ID NO:8) or an active fragment thereof,
5 mAPO4-αS (SEQ ID NO:10) or an active fragment thereof,
hAPO4-α (SEQ ID NO:12) or an active fragment thereof,
rAPO4-α (SEQ ID NO:14) or an active fragment thereof or
mAPO4-β (SEQ ID NO:16) or an active fragment thereof, or
mAPO4-γ (SEQ ID NO:32) or an active fragment thereof. An
10 APO4 antisense nucleic acid molecule, which has a
nucleotide sequence that is identically or substantially
complementary to an APO4 encoding nucleic acid or active
fragment thereof, also can be used to treat or reduce the
severity of an APO4-mediated disease according to a
15 method of the invention.

        The present invention also provides a method of
treating or reducing the severity of an APO4-mediated
disease in a subject such as, for example, prostate
cancer, by administering an APO4 regulatory agent to the
20 subject. As used herein, an "APO4 regulatory agent" is
an agent that inhibits or enhances a biological activity
of an APO4 polypeptide. Such an APO4 regulatory agent
can be, for example, an inhibitory agent capable of
inhibiting the activity of APO4 or an APO4-mediated
25 activity. For example, in the case of prostate cancer,
individuals with this disease can be treated with
inhibitors of a JNK pathway to reduce the severity of the
disease.

        An APO4 regulatory agent can also be an APO4
30 inhibitory agent such as a dominant negative form of
APO4, or an APO4 selective binding agent that inhibits a
biological activity of an APO4 polypeptide. Dominant
negative forms of APO4 include, for example, soluble

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forms of APO4 having an APO4 ligand binding domain such
as mAPO4-β, as well as membrane bound forms of APO4
having an APO4 ligand binding domain but lacking a
functional cytoplasmic tail such as mAPO4-αS, known as
5   "decoy receptors." One skilled in the art understands
that such an APO4 regulatory agent can be an agent that
selectively regulates a biological activity of an APO4
polypeptide or, alternatively, can be a non-selective
agent that, in addition to regulating a biological
10 activity of an APO4 polypeptide, also regulates the
activity of, for example, other TNF family receptors.

        As disclosed herein, amino acids 194 to 355 are
important for activation of the JNK pathway and
apoptosis. Since the mAPO4α-short and mAPO4-γ forms lack
15 the above domains, these and similar isoforms of APO4 can
lack the ability to induce the JNK pathway or cell death.
However, since these isoforms of APO4 possess a complete
or almost complete ligand-binding extracellular domain,
they can still compete with the full-length form of APO4
20 for binding to the ligand, thereby blocking signal
transduction mediated by the full-length receptor. As
such, mAPO4α-short, mAPO4-γ, their species homologs and
isoforms, and natural or synthetic compounds mimicking
their ligand-binding ability but lacking the complete
25 cytoplasmic domain or the domain encoding the amino acids
194 to 355 (or homologous domains in other species) can
be useful therapeutic tools for the treatment of diseases
resulting from the dysregulation of APO4 signal
transduction pathway. Such agents also can serve as lead
30 compounds for the development of therapeutic compounds
useful in the diagnosis and treatment of diseases
resulting from the dysregulation of APO4 function.

The APO6 encoding nucleic acids and
polypeptides of the invention can be used to diagnose,
treat or reduce the severity of an APO6-mediated disease.
For example, the APO6 encoding nucleic acids,
5   polypeptides and active fragments thereof can be used for
diagnosis of an APO6-mediated disease or can be used to
generate reagents useful for such diagnosis. Diagnosis
can be performed, for example, by nucleic acid probe
hybridization, or by amplification of APO6 encoding
10 nucleotide sequences and subsequent electrophoretic
analysis such as DNA sequencing. Diagnosis also can be
performed, for example using antibody or ligand based
detection with an APO6 selective binding agent.
Detection can be performed *ex vivo*, for example, by
15 removing a cell or tissue sample from an individual at
risk of, suspected of having or exhibiting one or more
symptoms of an APO6-mediated disease. Altered APO6
expression or activity is indicative of an APO6-mediated
disease. As used herein, the term "APO6-mediated
20 disease" means a disease, pathology, or other condition
resulting from an abnormality in the expression or
activity of an APO6 polypeptide. Included within the
meaning of the term "APO6-mediated disease" are disorders
of cell loss, disorders of cell accumulation and
25 disorders of cell differentiation.

The invention provides a method of treating or
reducing the severity of an APO6-mediated disease in a
subject by administering an APO6 polypeptide or active
fragment thereof or by administering a nucleic acid
30 molecule encoding the APO6 polypeptide or fragment. An
APO6 polypeptide or nucleic acid molecule useful in such
a method can be or encode, for example, an amino acid
sequence identical or substantially the same as hAPO6
(SEQ ID NO:18), or an active fragment thereof. An APO6

antisense nucleic acid molecule, which has a nucleotide
sequence that is identically or substantially
complementary to an APO6 encoding nucleic acid or active
fragment thereof, also can be used to treat or reduce the
5 severity of an APO6-mediated disease according to a
method of the invention.

In addition, the invention provides a method of
treating or reducing the severity of an APO6-mediated
disease in a subject by administering an APO6 regulatory
10 agent to the subject. An "APO6 regulatory agent," as
used herein, is an agent that inhibits or enhances a
biological activity of an APO6 polypeptide. Such an APO6
regulatory agent can be, for example, an APO6 inhibitory
agent such as a dominant negative form of APO6, an
15 alternatively spliced full-length form of APO6
polypeptide or an APO6 selective binding agent that
inhibits a biological activity of an APO6 polypeptide.
An APO6 regulatory agent also can be an agent that
selectively binds an alternatively spliced full-length
20 form of APO6, thereby blocking or transmitting a signal
through full-length APO6. One skilled in the art
understands that an APO6 regulatory agent can be an agent
that selectively regulates a biological activity of an
APO6 polypeptide or, alternatively, can be a
25 non-selective agent that, in addition to regulating a
biological activity of an APO6 polypeptide, also
regulates the activity of, for example, other TNF family
receptors.

The TNRL1 encoding nucleic acids and
30 polypeptides of the invention can be used to diagnose,
treat or reduce the severity of a TNRL1-mediated disease.
For example, the TNRL1 encoding nucleic acids,
polypeptides and active fragments thereof can be used for

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diagnosis of a TNRL1-mediated disease or can be used to
generate reagents useful for such diagnosis. Diagnosis
can be performed, for example, by nucleic acid probe
hybridization, or by amplification of TNRL1 encoding
5   nucleotide sequences and subsequent electrophoretic
analysis such as DNA sequencing. Diagnosis also can be
performed using, for example, antibody or ligand based
detection with a TNRL1 selective binding agent.
Detection can be performed *ex vivo*, for example, by
10   removing a cell or tissue sample from an individual at
risk of, suspected of having or exhibiting one or more
symptoms of a TNRL1-mediated disease. Altered TNRL1
expression or activity is indicative of a TNRL1-mediated
disease. As used herein, the term "TNRL1-mediated
15   disease" means a disease, pathology, or other condition
resulting from an abnormality in the expression or
activity of a TNRL1 polypeptide. Included within the
meaning of the term "TNRL1-mediated disease" are
disorders of cell loss, disorders of cell accumulation
20   and disorders of cell differentiation.

     The invention also provides a method of
treating or reducing the severity of a TNRL1-mediated
disease in a subject by administering a TNRL1 polypeptide
or active fragment thereof or by administering a nucleic
25   acid molecule encoding the TNRL1 polypeptide or fragment.
A TNRL1 polypeptide or nucleic acid molecule useful in
the method can be or encode, for example, an amino acid
sequence identical or substantially the same as hTNRL1-α
(SEQ ID NO:20) or an active fragment thereof, mTNRL1-α
30   (SEQ ID NO:22) or an active fragment thereof, hTNRL1-β
(SEQ ID NO:24) or an active fragment thereof, or mTNRL1-β
(SEQ ID NO:26) or an active fragment thereof. A TNRL1
antisense nucleic acid molecule, which has a nucleotide
sequence that is identically or substantially

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complementary to a TNRL1 encoding nucleic acid or active
fragment thereof, also can be used to treat or reduce the
severity of a TNRL1-mediated disease.

In addition, the invention provides a method of
5 treating or reducing the severity of a TNRL1-mediated
disease in a subject by administering a TNRL1 regulatory
agent to the subject. A "TNRL1 regulatory agent," as
used herein, is an agent that inhibits or enhances a
biological activity of a TNRL1 polypeptide. A TNRL1
10 regulatory agent can be, for example, a TNRL1 stimulatory
agent such as a soluble form of TNRL1 having a TNRL1
receptor binding domain. A TNRL1 regulatory agent also
can be, for example, a TNRL1 selective binding agent that
inhibits a biological activity of a TNRL1 polypeptide.
15 Such a TNRL1 selective binding agent can be, for example,
a small peptide, organic molecule or other agent which
competes with TNRL1 for binding to a TNRL1 receptor but
which, when bound, does not lead to receptor aggregation
or signal transduction. One skilled in the art
20 understands that a TNRL1 regulatory agent can be an agent
that selectively regulates a biological activity of a
TNRL1 polypeptide or, alternatively, can be a
non-selective agent that, in addition to regulating a
biological activity of a TNRL1 polypeptide, also
25 regulates the activity of, for example, other TNF family
ligands.

The TNRL3 encoding nucleic acids and
polypeptides of the invention also can be used to
diagnose, treat or reduce the severity of a
30 TNRL3-mediated disease. For example, the TNRL3 encoding
nucleic acids, polypeptides and active fragments thereof
can be used for diagnosis of a TNRL3-mediated disease or
can be used to generate reagents useful for such

diagnosis. Diagnosis can be performed, for example, by
nucleic acid probe hybridization, or by amplification of
TNRL3 encoding nucleotide sequences and subsequent
electrophoretic analysis such as DNA sequencing.
5 Diagnosis also can be performed, for example, using
antibody or ligand based detection with a TNRL3 selective
binding agent. Detection can be performed *ex vivo*, for
example, by removing a cell or tissue sample from an
individual at risk of, suspected of having or exhibiting
10 one or more symptoms of a TNRL3-mediated disease.
Altered TNRL3 expression or activity is indicative of a
TNRL3-mediated disease. As used herein, the term
"TNRL3-mediated disease" means a disease, pathology, or
other condition resulting from an abnormality in the
15 expression or activity of a TNRL3 polypeptide. Included
within the meaning of the term "TNRL3-mediated disease"
are disorders of cell loss, disorders of cell
accumulation and disorders of cell differentiation.

        The invention also provides a method of
20 treating or reducing the severity of a TNRL3-mediated
disease in a subject by administering a TNRL3 polypeptide
or active fragment thereof or by administering a nucleic
acid molecule encoding the TNRL3 polypeptide or fragment.
A TNRL3 polypeptide or nucleic acid molecule useful in
25 the method can be or encode, for example, an amino acid
sequence identical or substantially the same as hTNRL3
(SEQ ID NO:28) or an active fragment thereof, or mTNRL3
(SEQ ID NO:30) or an active fragment thereof. A TNRL3
antisense nucleic acid molecule, which has a nucleotide
30 sequence that is identically or substantially
complementary to a TNRL3 encoding nucleic acid or active
fragment thereof, also can be used to treat or reduce the
severity of a TNRL3-mediated disease.

In addition, the invention provides a method of
treating or reducing the severity of a TNRL3-mediated
disease in a subject by administering a TNRL3 regulatory
agent to the subject. A "TNRL3 regulatory agent," as
5 used herein, is an agent that inhibits or enhances a
biological activity of a TNRL3 polypeptide. A TNRL3
regulatory agent can be, for example, a TNRL3 stimulatory
agent such as a soluble form of TNRL3 having a TNRL3
receptor binding domain. A TNRL3 regulatory agent also
10 can be, for example, a TNRL3 selective binding agent that
inhibits a biological activity of a TNRL3 polypeptide.
Such a TNRL3 selective binding agent can be, for example,
a small peptide, organic molecule or other agent which
competes with TNRL3 for binding to a TNRL3 receptor but
15 which, when bound, does not lead to receptor aggregation
or signal transduction. One skilled in the art
understands that such a TNRL3 regulatory agent can be an
agent that selectively regulates a biological activity of
a TNRL3 polypeptide or, alternatively, can be a
20 non-selective agent that, in addition to regulating a
biological activity of a TNRL3 polypeptide, also
regulates the activity of, for example, other TNF family
ligands.

The present invention also provides a method of
25 treating or reducing the severity of a disorder of cell
loss. Examples of such pathologies, which can result
from excessive programmed cell death, include AIDS and
degenerative disorders such as Alzheimer's disease,
Parkinson's disease, amyotrophic lateral sclerosis,
30 retinitis pigmentosa, and cerebellar degeneration.
Disorders of cell loss caused by excessive programmed
cell death also include myelodysplastic syndromes such as
aplastic anemia and ischemic injuries such as myocardial
infarction, stroke and reperfusion injury. Any such

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pathology is encompassed by the term "disorder of cell
loss," which, as used herein, means a disease, pathology
or other condition characterized by excessive cell death
or differentiation or inadequate cellular proliferation
5   resulting in an abnormally reduced number of cells.

        Thus, the invention provides a method of
treating or reducing the severity of a disorder of cell
loss in a subject by administering an agent that
selectively decreases APO8 apoptotic activity, thereby
10 inhibiting apoptosis in the subject. The invention also
provides a method of treating or reducing the severity of
a disorder of cell loss in a subject by administering an
agent that selectively decreases apoptotic activity,
where the apoptotic activity is selected from the group
15 selected from APO9 apoptotic activity, APO4 apoptotic
activity or APO6 apoptotic activity. Such methods can be
used to treat, for example, acquired immunodeficiency
syndrome, which is characterized by an increased level of
apoptosis in an infected T cell as compared to a normal T
20 cell. The methods of the invention for treating or
reducing the severity of a disorder of cell loss in a
subject also can be used to treat neurodegenerative
disorders such as Alzheimer's disease or Parkinson's
disease, the disorders of cell loss described above or
25 another disorder of cell loss known in the art.

        As used herein, an agent that "selectively
decreases APO8 apoptotic activity" produces a
substantially greater reduction in the apoptotic activity
of an APO8 polypeptide relative to any reduction in the
30 apoptotic activity of another polypeptide, for example,
another pro-apoptotic TNF receptor such as TNFR-1.
Similarly, an agent that selectively decreases APO9, APO4
or APO6 apoptotic activity produces a substantially

greater reduction in the apoptotic activity of an APO9
polypeptide, an APO4 polypeptide, or an APO6 polypeptide,
respectively, relative to any reduction in the apoptotic
activity of an unrelated polypeptide.

5   The present invention also provides a method of
treating or reducing the severity of a disorder of
increased cell accumulation. Disorders of increased cell
accumulation include cancers such as lymphomas,
carcinomas, and hormone dependent tumors such as breast,
10 prostrate and ovarian tumors. Additionally, autoimmune
diseases, such as myasthenia gravis, rheumatoid
arthritis, systemic lupus erythematosus and
immune-mediated glomerulonephritis, and viral infections
such as herpesvirus, poxvirus and adenovirus can be a
15 disorder of increased cell accumulation. Pathological
conditions involving smooth or cardiac muscle cells, such
as hepatic necrosis vasculitis, angiogenesis,
atherosclerosis and myocarditis, also can result from
increased cell accumulation. Any such pathology is
20 encompassed within the term "disorder of increased cell
accumulation," which, as used herein, means a disease,
pathology or other condition characterized by diminished
cell death or differentiation or excess cell
proliferation resulting in an abnormally high number of
25 cells.

           Provided herein is a method of treating or
reducing the severity of a disorder of cell accumulation
in a subject by administering an APO8 polypeptide or
active fragment thereof, or a nucleic acid molecule
30 encoding the APO8 polypeptide or active fragment, to the
subject. The present invention also provides a method of
treating or reducing the severity of a disorder of cell
accumulation in a subject by administering an APO9

polypeptide or active fragment thereof, or a nucleic acid
molecule encoding the APO9 polypeptide or fragment, to
the subject. Further provided herein is a method of
treating or reducing the severity of a disorder of cell

5   accumulation in a subject by administering an APO4
polypeptide or active fragment thereof, or a nucleic acid
molecule encoding the APO4 polypeptide or fragment, to
the subject. The invention also provides a method of
treating or reducing the severity of a disorder of cell

10   accumulation in a subject by administering an APO6
polypeptide or active fragment thereof, or a nucleic acid
molecule encoding the APO6 polypeptide or fragment, to
the subject.

         The TNRL1 and TNRL3 polypeptides of the

15   invention also can be useful in treating or reducing the
severity of the disorder of cell accumulation. The
present invention provides a method of treating or
reducing the severity of a disorder of cell accumulation
in a subject by administering a TNRL1 polypeptide or

20. active fragment thereof, or a nucleic acid molecule
encoding the TNRL1 polypeptide or fragment, to the
subject. Also provided herein is a method of treating or
reducing the severity of a disorder of cell accumulation
in a subject by administering a TNRL3 polypeptide or

25   active fragment thereof, or a nucleic acid molecule
encoding the TNRL3 polypeptide or fragment, to the
subject.

         Further provided herein is a method of treating
or reducing the severity of a disorder of cell

30   accumulation in a subject by administering an agent that
selectively increases APO8 apoptotic activity, thereby
enhancing apoptosis in the subject. The invention also
provides a method of treating or reducing the severity of

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a disorder of cell accumulation in a subject by
administering an agent that selectively increases
apoptotic activity, where the apoptotic activity is
selected from the group consisting of APO9 apoptotic
5   activity, APO4 apoptotic activity or APO6 apoptotic
activity. The present invention further provides a
method of treating or reducing the severity of a disorder
of cell accumulation in a subject by administering an
agent that selectively increases apoptotic activity,
10   where the apoptotic activity is selected from the group
consisting of TNRL1 apoptotic activity and TNRL3
apoptotic activity. The methods of the invention for
treating or reducing the severity of a disorder of cell
accumulation can be used to treat, for example, cancers
15   such as breast, prostrate and ovarian tumors, lymphomas
and carcinomas as well as the disorders of cell
accumulation described above or another disorder of cell
accumulation known in the art.

As used herein, an agent that "selectively
20   increases APO8 apoptotic activity" means an agent that
produces a substantially greater enhancement of the
apoptotic activity of an APO8 polypeptide relative to any
enhancement of the apoptotic activity of another
polypeptide, for example, another pro-apoptotic TNF
25   receptor such as CD27. Similarly, an agent that
selectively increases APO9, APO4, or APO6 apoptotic
activity produces a substantially greater enhancement of
the apoptotic activity of an APO9 polypeptide, an APO4
polypeptide, or an APO6 polypeptide, respectively,
30   relative to any enhancement of the apoptotic activity of
an unrelated polypeptide. In addition, an agent that
selectively increases TNRL1 or TNRL3 apoptotic activity
produces a substantially greater enhancement of the
apoptotic activity of a TNRL1 polypeptide or TNRL3

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polypeptide, respectively, relative to any enhancement of an unrelated polypeptide such as TNF-α.

The following examples are intended to illustrate but not limit the present invention.

5   **EXAMPLE I**

Identification and Characterization of human APO8 and APO8 related polypeptide

This example describes the identification and characterization of nucleic acid sequences encoding
10   full-length human APO8 and the APO8 related polypeptide APO8RP.

Isolation and characterization of the hAPO8 cDNA

APO8 is a human cDNA related to IMAGE consortium clones 650744 and 664665, which were derived
15   from a Stratagene NT neuronal library. These clones were identified by searching the EST database (dbEST) for sequences with homology to the extracellular domain of human TNFR-1 protein using the TBLASTN algorithm 5' and 3' RACE was performed on human fetal brain Marathon-ready
20   mRNA (Clontech, Palo Alto, CA) according to the manufacturer's instructions. 3' RACE was performed with primer SEQ ID NO:34 (5'-CTGTTGGATCCAGCTGAGTCTGCTCTGATCACCCAAC-3') and AP1 primer SEQ ID NO:38 (5'-CCATCCTAATACGACTCATATAGGGC-3').
25   The subsequent round of nested PCR was performed with primer SEQ ID NO:35 (5'-GGACACCATATCTCAGAAGACGGTAGAG-3') AP2 primer SEQ ID NO:36 (5'-ACTCATATAGGGCTCGAGCGGC-3'). 5' RACE was performed with primer SEQ ID NO:37 (5'-TATAGTCCTGTCCATATTTGCAGGAGATG-3') and the AP1 primer
30   SEQ ID NO:38. The subsequent round of nested PCR was

performed with primer SEQ ID NO:38
(5'-CTCTACCGTCTTCTGAGATATGGTGTCC-3') and the AP2 primer
SEQ ID NO:36. RACE fragments were cloned into the PCR2.1
vector using the Original TA Cloning kit (Invitrogen,
5   Carlsbad, CA). Inserts from eight or more individual
clones were PCR amplified using flanking vector primers
Vn26 (SEQ ID NO:39; 5'-TTTCCCAGTCACGACGTTGTA-3') and Vn27
(SEQ ID NO:40; 5'-GTGAGCGGATAACAATTTCAC-3') and purified
using the Wizard PCR kit (Promega, Madison, WI). The
10 inserts were sequenced on an ABI 373 automated
fluorescent sequencer with dye terminators using purified
PCR product or plasmid DNA as template and primers SEQ ID
NOS:39 and 40 as the sequencing primers. Percentage
identity and similarity scores were obtained using the
15 Gap program (Genetic Computer Group, Madison, WI).

        The isolated full-length APO8 cDNA nucleotide
sequence, provided as SEQ ID NO:1, encodes a polypeptide
of 411 amino acids with characteristics of a cell surface
receptor, including an N-terminal signal peptide of 51
20 amino acids and a transmembrane region of 27 amino acids
(residues 185 to 212). The context of the initiating
methionine (CCGCCATGG) conforms to the Kozak consensus
sequence, and an in-frame stop codon ten amino acids
upstream of this methionine confirmed that it is the true
25 start site. The extracellular domain (residues 52
to 184) of hAPO8 contains the cysteine-rich
pseudo-repeats that are a hallmark of the TNF receptor
family and exhibits a high degree of amino acid sequence
homology to the TRAIL receptor (DR4) (58% identity and
30 70% similarity; Pan et al., supra, 1997, which is
incorporated herein by reference). To a lesser extent,
the extracellular domain of hAPO8 is homologous to TNFR-1
(27% amino acid identity and 46% similarity), FAS/APO-1
(25% amino acid identity and 45% similarity), DR3 (21%

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amino acid identity and 40% similarity) and other members
of the TNFR family (see Table 1). Based on this
homology, the APO8 polypeptide is classified as a new
member of the TNFR family.

5   hAPO8 has a cytoplasmic tail of 212 amino acids
(residues 213 to 411) with a death domain near the
C-terminus. As shown in Figure 4B, the APO8 death domain
exhibits significant sequence homology to the death
domains of DR4, TNFR-1, FAS/APO-1 and DR3. Furthermore,
10   several amino acids that are essential for transmission
of the apoptotic signal by TNFR-1 and FAS/APO-1 also are
conserved in the death domain of APO8. This includes
leucine residue 334, which corresponds to the site of the
lpr mutation in the murine FAS receptor (see Figure 4B).

15   Expression of hAPO8 was studied by Northern
analysis. A major transcript of about 4.4 kb was seen in
all tissues tested, including spleen, thymus, prostate,
testis, ovary, small intestine, colonic mucosa and
peripheral blood lymphocyte (PBL), with the highest level
20   of expression in PBL. A more slowly migrating minor
transcript, which may represent the product of
alternative splicing, was present in all tissues assayed.
These results demonstrate that RNA encoding the human
APO8 polypeptide is widely expressed.

25   Northern analysis was performed using a
multiple human tissue RNA blot (#7759-1) from Clontech.
The blot was hybridized with a ^{32}P-labeled APO8 cDNA
according to the manufacturer's instructions.

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Characterization of the APO8 related polypeptide (APO8RP)
cDNA

The APO8RP nucleotide sequence, shown in
Figure 3A, encodes a polypeptide of 410 amino acids.
5 Alignment of the amino acid sequences of human APO8 (SEQ
ID NO:2) and human APO8RP (SEQ ID NO:4) revealed that
hAPO8RP exhibits substantial homology to hAPO8 throughout
the entire polypeptide sequence. The hAPO8 and hAPO8RP
predicted signal peptides, transmembrane regions and
10 death domains are shown in Figure 4A. In particular, the
hAPO8RP signal peptide sequence (residues 1 to 51) is
homologous to the hAPO8 signal peptide (residues 1
to 51), indicating that the N-terminal portion of the
hAPO8RP signal is Met-Gly-Gln-His. Hydrophobicity plots
15 of the hAPO8 and hAPO8RP sequences support the conclusion
that the hAPO8 signal peptide corresponds to residues 1
to 51 shown in Figure 4A and that the APO8RP signal
peptide corresponds to residues 1 to 51 shown in
Figure 4A. Furthermore, two artificial neural networks
20 (Nielsen et al., Protein Eng. 10:1-6 (1997), which is
incorporated by reference herein), trained at recognizing
eukaryotic signal peptides and their cleavage sites,
identified cleavable signal peptides between amino acids
1 to 51 of APO8 and between amino acids 1 to 51 of
25 hAPO8RP, respectively. The results with hAPO8RP contrast
with those of Pan et al., *supra,* 1997, which describe a
related protein containing an additional 58 N-terminal
residues.

Hydrophobicity plots were generated by using
30 the Pepplot program (Genetic Computer Group). Artificial
neural networks analysis for recognizing the signal
peptides and their cleavage sites was performed using the

Signal V1.1 World Wide Web Server (http://www.cbs.dtu.dk/service

EXAMPLE II

APO8 and DR4 regulate NF-κB expression and induce
FADD-dependent apoptosis

5   This example demonstrates that APO8 and APO8RP
induce cell death through the cytoplasmic protein FADD.
In addition, the APO8 and APO8RP polypeptides activate
NF-κB expression.

APO8 induces apoptosis

10   The ability of hAPO8 to induce apoptosis was
assayed in the human breast carcinoma cell line MCF-7,
the human embryonic kidney cell 293T subclone and the
baby hamster kidney BHK cell line. Transient
transfection of full-length hAPO8 induced rapid apoptosis
15   in all three cell lines tested. In contrast, expression
of an APO8 deletion mutant lacking the C-terminal 192
amino acids ("APO8ΔCP") or an APO8 deletion mutant
lacking the C-terminal 134 amino acids, which includes
the death domain, ("APO8ΔDD") failed to induce apoptosis.
20   Exemplary results in MCF-7 cells are shown in Figure 5A.
These data demonstrate that the C-terminal 134 amino
acids of hAPO8 including its death domain are essential
for transmitting the apoptotic signal. Furthermore, a
mutant construct containing a leucine to asparagine
25   substitution at residue 334 ("APO8-L334N") also failed to
induce apoptosis (Figure 4A). Leucine 334 corresponds to
the site of the *lpr* mutation and is essential for
apoptotic signaling in TNFR-1, FAS/APO-1 and DR3. These
results indicate that, like TNFR-1, FAS/APO-1 and DR3,
30   leucine residue 334 is required for apoptotic activity of
hAPO8.

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APO8 and APO8RP induced apoptosis is mediated by
FADD/MORT1

The adaptor molecule FADD/MORT1 mediates
apoptosis by the death domain containing receptors
5   TNFR-1, FAS/APO-1 and DR3 either directly or through an
intermediate adaptor molecule TRADD. For example,
dominant negative FADD can effectively block the
apoptosis mediated by these death receptors. A dominant
negative FADD construct consisting of the FADD death
10   domain was tested for the ability to modulate APO8 and
APO8RP mediated apoptosis in 293T cells. Increasing
amounts of dominant negative FADD led to progressive
inhibition of apoptosis mediated by DR3, which has
previously been shown to mediate cell death through FADD.
15   As shown in Figure 5B, progressive inhibition of
apoptosis was also observed for the hAPO8 and hAPO8RP
receptors, with almost complete inhibition of apoptosis
at a receptor to dominant negative FADD DNA ratio of
1:10. Dominant negative FADD also inhibited APO8
20   mediated apoptosis in MCF-7 cells (Figure 5C). These
results demonstrate that both hAPO8 and hAPO8RP induced
apoptosis is dependent upon FADD/MORT. Thus, a variety
of death domain containing receptors (TNFR-1, FAS/APO-1,
DR3, hAPO8 and hAPO8RP) induce apoptosis through the
25   FADD/MORT adaptor molecule.

Apoptotic signaling by the TNF receptors TNFR-1
and DR3 involves FADD-mediated recruitment of the ICE
protease FLICE/MACH1 (Caspase 8) to the aggregated death
receptor complex. To test whether hAPO8 induced
30   apoptosis also involves recruitment of FLICE/MACH1, the
ability of a dominant negative FLICE mutant to block
APO8-induced apoptosis was tested. This FLICE mutant
contains a cysteine to serine substitution at the

catalytic site and is designated "FLICE-C360S." As shown
in Figure 5C, this FLICE-C360S mutant was able to
effectively block apoptosis mediated by APO8. In view of
previous results demonstrating that FLICE interacts with
5 FADD, these data provide further evidence that
APO8-induced apoptosis relies on a FADD-dependent
pathway.

Several caspase inhibitors, z-VAD-fmk and CrmA,
have previously been shown to block apoptosis of TNF
10 death receptors. The ability of these caspase inhibitors
to effect APO8 mediated apoptosis was assayed, and the
results are shown in Figure 5C. These results
demonstrate that z-VAD-fmk and CrmA each inhibit
APO8-induced apoptosis, indicating that APO8 causes cell
15 death through a caspase-dependent pathway.

Myc and 6X-His epitope tagged APO8 constructs
were prepared by amplifying amino acids 51 to 411 of APO8
using *pfu* polymerase (Stratagene, La Jolla, CA) with a 5'
primer containing a BamHI site and a 3' primer containing
20 a SalI site. The amplified APO8 fragment was
subsequently ligated to a modified pSecTag A vector
(Invitrogen) containing a Myc or an MRG-6XHis epitope tag
downstream of a murine Ig κ-chain signal peptide. The
APO8-L334N construct, which has a leucine to asparagine
25 mutation at residue 334, was generated by mutagenesis of
the 6X-His-APO8 plasmid with primers SEQ ID NO:41
(5'-CGCTCATGAGGAAGTTGGGCAACATGGACAATGAGATAAAG-3') and SEQ
ID NO:42(5'-CTTTATCTCATTGTCCATGTTGCCCAACTTCCTCATGAGCG-3')
using the Quickchange kit (Stratagene) according to the
30 manufacturer's instructions. Deletion construct APO8ΔCP
lacks the 192 C-terminal residues of full-length APO8 and
was constructed by creating a PCR generated XbaI site in
the wild type APO8 sequence. Deletion construct APO8ΔDD

lacks the C-terminal 134 residues of APO8. and was
prepared using a naturally occurring SmaI site in the
APO8 sequence. The sequences of each of the above
constructs were confirmed by automated fluorescent
5 sequencing.

         Epitope-tagged versions of APO8RP (amino acids
52 to 410), DR3 (amino acids 27 to 418) and FAS/APO1
(amino acids 19 to 335) were constructed as described
above for the APO8 expression vector except that the 5'
10 primer for amplifying Fas/APO1 contained an EcoRI site
and the resulting PCR product was cloned into the EcoRI
and SalI sites of the modified pSecTag A vector.

         The dominant negative FADD construct (DN-FADD)
encodes amino acids 80 to 208 of human FADD in pCDNA3
15 (Invitrogen) and was prepared by Michael Wright of the
University of Washington. The FLICE-C360S mutant, which
contains a cysteine to serine substitution at residue
360, was prepared with primers SEQ ID NO:43
(5'-GTGTTTTTTATTCAGGCTAGTCAGGGGGATAACTACCAGAA-3') and SEQ
20 ID NO:44 (TTCTGGTAGTTATCCCCCTGACTAGCCTGAATAAAAAACAC-3')
using Stratagene's Quickchange kit. The N-terminal TRADD
deletion mutant ND-TRADD-HA, which lacks the N-terminal
102 amino acids, and RIP-HA tagged constructs were
prepared using custom primers designed to generate a
25 C-terminal HA-tag. The sequence of each of the above
constructs was confirmed by automated fluorescent
sequencing.

         For transient transfection assays, 0.8-1 X 10^5
MCF-7 or BHK cells were cotransfected with 1 μg of
30 various test plasmids and 200 ng of a lacZ expression
plasmid in a 24-well plate using 3 μl of Superfect
(Qiagen, Santa Clarita, CA) in DMEM supplemented with 10%

FCS essentially according to the manufacturer's
instructions. After 3 hr, 0.5 ml of fresh DMEM
supplemented with 10% FCS was added. Twenty-four to
thirty-six hours later, cells were fixed with 0.05%
5   glutaraldehyde in PBS and stained with
5-bromo-4-chloro-3-indolyl-β-D-galactosidase (X-GAL).
For transient transfection of 293T cells, 1 x 10^5 cells
were transfected by calcium phosphate precipitation in a
24-well plate essentially as described in Sambrook et
10 al., *supra,* 1989. The data shown in Figure 5A represent
the mean +/- the standard deviation determined from three
independent experiments performed in duplicate. The
percentage of apoptotic cells was calculated as the
percentage of cells showing β-galactosidase activity and
15 apoptotic morphology as indicated by a round and
condensed shape as compared to the total number of blue
staining cells.

For the dominant negative FADD experiments
shown in Figure 5B, 150 ng of each receptor (APO8, APO8RP
20 or DR3) was transfected into 293T cells with 150 ng, 750
ng or 1500 ng of DN-FADD DNA. The total amount of DNA in
each transfection was held constant by adding control
vector plasmid DNA. The results shown in Figure 5B are
representative of two independent experiments performed
25 in duplicate.

For the inhibition experiments shown in
Figure 5C, MCF-7 cells were transfected with the Myc-APO8
expression construct and a control empty expression
vector or an expression vector encoding DN-FADD,
30 FLICE-C360S or CrmA. For experiments with the protease
inhibitor z-VAD-fmk, cells transfected with Myc-APO8 and
the control vector were treated with 20 μM z-VAD-fmk
twelve hours after transfection. Cells were transfected

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and scored for apoptotic morphology as described above.
The data shown in Figure 5C are representative of three
independent experiments performed in duplicate.

APO8 and APO8RP activate NF-κB by a TRADD dependent
5   pathway

Activation of TNFR-1 and DR3 has been shown to
induce NF-κB expression, and NF-κB activation can block
apoptosis. To test the possibility that APO8 can
regulate NF-κB expression, APO8 expression constructs
10 were cotransfected into 293T cells with a NF-κB
luciferase reporter containing four copies of an NF-κB
binding site. As shown in Figure 5D, overexpression of
APO8 produced significant activation of NF-κB in 293T
cells, while C-terminal deletion mutants APO8ΔCP and
15 APO8ΔDD and the APO8-L334N point mutant did not activate
NF-κB. Similarly, overexpression of APO8RP activated
NF-κB as compared to control expression vector (see
Figure 5D). These results show that APO8 activates NF-κB
expression and that the APO8 death domain is required for
20 this activation. In addition, these results show that
APO8RP, in contrast to the DR4 receptor described in Pan
et al., *supra*, 1997, also activates NF-κB expression.

NF-κB activation experiments were performed as
follows using a NF-κB luciferase reporter with four NF-κB
25 binding sites and a minimal thymidine kinase promoter
(Berberich et al., J. Immunol. 153:4357-4366 (1994),
which is incorporated herein by reference). 293T cells
were cotransfected in duplicate as described above with
0.5 μg of Myc-tagged or 6XHIS-tagged receptor expression
30 construct, 0.5 μg NF-κB reporter construct and 0.2 μg
lacZ expression construct. After eighteen hours, cells
in one well were fixed and stained with X-GAL to

determine the relative transfection efficiency. Cell
extracts were prepared from the duplicate well and
analyzed for luciferase activity using the Luciferase
Assay system (Promega, Madison, WI) following the
5   manufacturer's instructions. Shown in Figure 5D is the
mean +/- standard deviation calculated from three
independent representative experiments.

***Dominant negative TRADD can block NF-κB activation by
APO8, APO8RP, and DR3***

10   As described above, the death domain of APO8 is
required for APO8-mediated apoptosis and for NF-κB
activation. TNFR-1 and DR3 mediated apoptosis and NF-κB
activation also are mediated through the TNFR-1 and DR3
death domains, and may be a result of death domain
15   recruitment of TRADD (Kitson et al., Nature 384:372-375
(1996); Tartaglia et al., Cell 74:845-853 (1993), each of
which is incorporated herein by reference). TRADD can
activate the apoptotic pathway through FADD and can
activate the NF-κB pathway through RIP and TRAF2 (Hsu et
20   al., Cell 84:299-308 (1996); Liu et al., Cell 87:565-576,
each of which is incorporated herein by reference).

            A TRADD mutant deleted for the N-terminal 102
residues (ND-TRADD) lacks a complete TRAF2-binding domain
but has a complete RIP/FADD-binding death domain (Liu et
25   al., *supra,* 1996). This TRADD mutant may function as a
dominant negative by competing with endogenous
full-length TRADD for binding to receptor death domains
but failing to effectively recruit TRAF2. The ability of
dominant negative TRADD (ND-TRADD) to block NF-κB
30   activation by APO8, APO8RP and DR3 was tested in 293T
cells.

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Briefly, 293T cells were cotransfected in
duplicate with an appropriate receptor and ND-TRADD
along with NF-κB (0.5 μg) and lacZ (0.2 μg) reporter
constructs. The receptor-to-ND-TRADD ratio was 1:5 (0.15
5   μg to 0.75 μg). The total amount of plasmid was kept
constant by adding vector DNA without insert. Eighteen
hours post transfection, luciferase activity was measured
from one of the duplicate wells using the luciferase
assay reagent (Promega) and following the manufacturer's
10 instructions. The cells in the other well were fixed
with glutaraldehyde and stained with X-gal to obtain the
relative transfection efficiency. Results (mean +/- SD)
are from at least four independent experiments.

The results showed that ND-TRADD effectively
15 blocked NF-κB activation by all three death domain
receptors. Weak NF-κB activation was observed with
ND-TRADD transfected alone in 293T cells, possibly as a
result of the ability of ND-TRADD to recruit RIP and,
indirectly, TRAF2 through death domain-death domain
20 interactions (Liu et al., *supra,* 1996). In sum, these
results indicate that APO8 and APO8RP resemble DR3 and
TNFR-1 in activating NF-κB through a TRADD-dependent
pathway.

APO8 and APO8RP interact directly with TRADD and RIP but
25 **not with FADD**

Coprecipitation experiments were conducted to
assay whether APO8 or APO8RP interacts directly with the
FADD, TRADD or RIP adaptor molecules. Under conditions
which supported the interaction of His epitope tagged
30 FAS/APO1 with FADD, both His tagged APO8 and APO8RP
failed to coprecipitate AU1-tagged FADD (AU1-FADD). In
contrast, HA-tagged ND-TRADD or RIP were coprecipitated

with APO8 or APO8RP. Furthermore, both APO8 and APO8RP
co-immunoprecipitated FADD in the presence of ND-TRADD.
Thus, these results show that both APO8 and APO8RP, like
DR3 and TNFR-1, directly bind TRADD and RIP and
5   indirectly bind FADD through TRADD. The results with
APO8RP are contrary to the results reported in Pan et
al., *supra*, 1997, with the DR4 receptor. In sum, FADD
appears to be the common mediator of apoptosis by all
known death domain-containing receptors.

10   Coimmunoprecipitation experiments between
receptors and various adaptor molecules were performed as
follows. 293T cells (2 x 10^6) were plated in a 10 mm
plate and cotransfected the following day with 5 μg of
various epitope tagged receptor plasmids or control
15   vector lacking receptor sequence; 5 μg of various adaptor
plasmids; 2 μg of Crm-A plasmid; and 0.5 μg of a Green
Fluorescent Protein (GFP) encoding plasmid (pEGFP-N1,
Clontech) by calcium phosphate coprecipitation. The
following day, cells were examined under a fluorescent
20   microscope to ensure equal transfection efficiency as
determined by the expression of the GFP. Twenty-four to
thirty-six hours post-transfection, cells were lysed in 1
ml of lysis buffer containing 1% Triton X-100, 20 mM
sodium phosphate (pH 7.4), 150 mM NaCl and 1 EDTA-free
25   protease inhibitor tablet per 10 ml (Boehringer Mannheim,
Indianapolis, IN), and the cell lysate cleared by
centrifugation.

        For immunoprecipitation, pre-cleared cells
lysate (600 μl) was incubated with 2 μg of anti-Myc
30   monoclonal 9E10 (American Type Culture Collection,
Rockville, MD) for 2 hr at 4°C. The lysate was
subsequently incubated for 1 hour with 10μl of Protein
A/G ultralink beads (Pierce, Rockford, IL) pre-coated

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with 1% BSA to reduce nonspecific binding. Beads were
washed twice with lysis buffer, followed by two washes in
buffer containing 1% Triton X-100, 20 mM sodium phosphate
(pH 7.4) and 500 mM NaCl and once with a buffer
5   containing 20 mM Tris-HCl (pH 7.4). Bound proteins were
eluted by adding 70 μl of 0.1 M glycine (pH 3.5) and
subsequently neutralized with 10μl of 1 M Tris-HCl (pH
8.0). Samples were separated by SDS-PAGE, transferred to
a nitrocellulose membrane and analyzed by Western blot
10 analysis. For immunoprecipitation of MRG-6XHis tagged
receptors, a cocktail of three monoclonal antibodies
(MRG-4XHis, Penta-His and Tetra-His; Qiagen) was used.
For precipitation using Nickel-sepharose, cell lysates
were incubated for 1 hour with 20μl of Ni-sepharose beads
15 pre-coated with 1% BSA in a buffer containing 1 % Triton
X-100, 20 mM sodium phosphate (pH 7.4), 500 mM NaCl and
EDTA-free protease inhibitor cocktail. Beads were washed
extensively with the above buffer supplemented with 20 mM
imidazole, and the bound protein subsequently eluted and
20 analyzed by SDS-PAGE and Western blot analysis as
described above.

NF-κB expression protects against APO8-mediated apoptosis

        293T cells were transfected with APO8
25 expression vector in the presence or absence of an NF-κB
expression vector. Thirty-six hours later, cells were
fixed and stained and morphology observed. Apoptotic
morphology was observed in about 55% of APO8-transfected
cells in the absence of NF-κB, while only about 30% of
30 APO8-transfected cells were apoptotic when co-transfected
with NF-κB. These results indicate that NF-κB protects
against APO8-mediated cell killing.

Cell transfections were performed as described
above using 1 x 10^5 293T cells and 150 ng APO8 expression
vector, 750 ng NF-κB expression vector or control empty
vector, and 200 ng lacZ encoding plasmid in 24 well
5   plates. After thirty-six hours, cells were fixed and
stained and the percent apoptotic cells determined as
described above.

**Myc-APO8RP is expressed on the cell surface and binds
TRAIL ligand**

10   The cytotoxic ligand TRAIL has been shown to
bind the DR4 receptor based on the ability of a soluble
DR4 construct to coimmunoprecipitate TRAIL and to block
TRAIL-induced apoptosis in MCF-7 cells. Direct binding
of APO8RP to TRAIL was assayed by preparing a stable BHK
15   cell line expressing Myc-epitope tagged APO8RP.
Cell-surface expression of APO8RP was confirmed by
immunofluorescence staining with an anti-Myc antibody
using FACS analysis. Subsequently, the ability of
FLAG-epitope tagged TRAIL (FLAG-TRAIL) to bind the APO8RP
20   transformant was tested using immunofluorescence staining
with an anti-FLAG antibody. The results demonstrated
that FLAG-TRAIL binds Myc-APO8RP. These results
demonstrate that APO8RP, like DR4, binds the TRAIL
ligand.

25   Epitope tagged TRAIL protein was prepared as
follows. A PCR fragment encoding amino acids 96 to 281
of TRAIL was cloned into a modified pET28(b) vector
(Novagen Madison, WI), which has a FLAG epitope
downstream of the His epitope, and the sequence confirmed
30   by automated fluorescent sequencing. The His-FLAG-TRAIL
protein was purified by Nickel-affinity chromatography
according to the manufacturer's instructions.

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Stable cell lines were generated by
cotransfecting 2 x 10⁵ BHK cells with 1.5 μg of a
Myc-epitope tagged APO8RP expression vector or control
vector lacking the receptor coding sequence and 300 ng of
5   an expression vector encoding human DHFR gene using
Superfect (Qiagen) according to the manufacture's
instructions. Stable transformants were selected in 1 μM
methotrexate (Sigma, St. Louis, MO) in DMEM supplemented
with 5% dialyzed fetal calf serum. After 10 to 12 days
10 colonies were pooled to generate a mass culture.

Expression of the Myc-APO8RP construct was
analyzed by FACS after indirect immunofluorescence
labeling using the anti-Myc epitope antibody mAb 9E10
obtained from American Type Culture Collection or a
15 control monoclonal antibody, followed by R-phycoerythrin
(R-PE) labeled goat anti-mouse antibody (Fisher).
Binding of FLAG-TRAIL (2 μg) to the Myc-APO8RP expressing
stable cell lines was detected similarly using the
anti-FLAG M2 antibody (IBI Kodak, New Haven, CT) as the
20 primary antibody and R-PE-labeled goat anti-mouse
antibody as the secondary antibody.

EXAMPLE III

Identification and Characterization of APO9

This example describes the identification and
25 characterization of alternatively spliced forms of APO9.

APO9 is a human cDNA related to IMAGE
consortium clones 470799, 504745, 110226 and 129137,
which are clones with homology to human Fas antigen. The
partial amino acid sequence of hAPO9S encodes a signal
30 peptide at the 5' end and a hydrophobic transmembrane
region at the 3' end as shown in Figure 6. In addition,

the hAPO9S sequence has a stop codon immediately after the transmembrane region. Thus, the hAPO9S cDNA can represent an alternatively spliced non-functional receptor isoform that competes with the full-length
5   receptor for ligand binding.

        In its extracellular domain, the hAPO9S amino acid sequence demonstrates significant homology to the extracellular domains of several members of the TNFR family. The greatest degree of homology is seen with the
10 DR4 receptor, with which it shares 57% amino acid sequence identity and 71% amino acid sequence similarity (see Figure 1 and Table 1). Based on this homology, the APO9S cDNA is classified as a new member of the TNFR family.
15

                            EXAMPLE IV
        Identification and Characterization of APO4 polypeptides

        This example describes the identification and characterization of nucleic acid sequences encoding the
20 short and long isoforms of murine, rat and human APO4-α and murine APO4-β. In addition, the ability of hAPO4 to induce cell death through activation of the JNK pathway and the ability of hAPO4 to activate NF-κB expression is demonstrated.

25 *Characterization of murine APO4-α and mAPO4-γ*

        mAPO4 are murine cDNAs related to IMAGE consortium EST clones 472300 and 427152, which are mouse embryo-derived sequences with homology to the human DR3 receptor. These clones were identified by searching the
30 EST database (dbEST) for homology to the extracellular domain of human DR3 using the TBLASTN algorithm.

The mAPO4-αS cDNA is represented by IMAGE consortium EST clone 472300 and has an open reading frame of 214 amino acids (Figure 7B), including an amino-terminal signal-peptide; cysteine-rich pseudo-
5 repeats with significant homology to other members of TNFR family (20-24% sequence identity and 35-46% sequence similarity); a highly hydrophobic stretch of amino-acids representing the transmembrane region; and a short cytoplasmic tail.

10   The mAPO4-γ cDNA is represented by the IMAGE consortium EST clone 427152 and has an open leading frame of 150 amino acids. The amino acid sequence of mAPO4-γ is identical to the mAPO4-α form with the N-terminal 149 amino acids containing the signal peptide and the
15 cysteine-rich pseudo repeats representing the majority of the ligand binding domain. However, mAPO4-γ has a stop codon after amino acid 150 and thus represents a soluble receptor lacking a transmembrane domain.

         No polyadenylation signal was observed at the
20 3' end of mAPO4-αS, indicating that the stop codon present in the 3' end can be the result of alternative splicing. 3' Rapid Amplification of cDNA Ends (RACE) was used on murine spleen marathon-ready cDNA to isolate the full length cDNA. The full length cDNA has an open
25 reading frame of 416 amino acids and a unique cytoplasmic tail with no significant homology to other members of TNF receptor family (Figure 7A). Thus, mAPO4-α is a type I membrane protein, having short and long forms. The long mAPO4-α form can have an important role in the regulation
30 of the immune system, while the short form of the receptor is a decoy receptor which can compete with the long (full-length) form for ligand binding, thereby

modulating signal-transduction through the full-length receptor.

The full-length mAPO4-α (mAPO4-αL) sequence was
obtained using 3' RACE and murine spleen Marathon-ready
cDNA (Clontech) according to the manufacturer's
5   instructions. Primer SEQ ID NO:45
(5'-CCCACCTCCTCCCTACGAACCACACTG-3') was used with AP1
primer SEQ ID NO:34 in a first round of PCR. Primer SEQ
ID NO:46 '-GGCTGCTCGCCCTGCTCATCCTGTGTGT-3') and AP2
primer SEQ ID NO:36 were used in a subsequent round of
10   nested PCR. RACE fragments were cloned into the PCR2.1
vector using the Original TA Cloning kit (Invitrogen).
Inserts from eight or more individual clones were PCR
amplified using flanking vector primers Vn26 (SEQ ID
NO:39; 5'-TTTCCCAGTCACGACGTTGTA-3') and Vn27 (SEQ ID
15   NO:40), and the amplified fragments purified using the
Wizard PCR Preps kit (Promega). The inserts were
sequenced on an ABI 373 automated fluorescent sequencer
with dye terminators using the purified PCR products or
plasmid DNA as template with the Vn26 (SEQ ID NO:39) and
20   Vn27 (SEQ ID NO:40) primers as sequencing primers.

mAPO4-αL is a cell surface receptor

To demonstrate that mAPO4-αL is a cell surface
receptor, Myc-AU1 tagged mAPO4-αL was expressed in BHK
cells. Cell surface expression was confirmed by
25   immunofluorescence labeling with the Myc monoclonal
antibody 9E10 (American Type Culture Collection). These
results show that mAPO4-αL is a cell surface receptor.

The Myc-AU1 tagged mAPO4-αL construct was
prepared by PCR amplifying the protein coding region of
30   the mature APO4-αL polypeptide with a 5' primer
containing a BamHI site and encoding the AU1 tag and a 3'

primer containing a XhoI site using *pfu* polymerase
(Stratagene). The 5' primer was SEQ ID NO:47
(5'-GGATCCAGACACATACCGCTACATTGCATGTAAAGTGAGTTGCGAAACC-3',
and the 3' primer was SEQ ID NO:48

5 (5'-GAAGCTCGAGGCTGAAAGTATGGAAGTGCTG-3'). The resulting
amplified product was digested with BamHI and XhoI and
ligated to a BamHI/XhoI digested pSecTag A vector
modified to include a Myc tag as described above.

Characterization of human APO4-*α*

10   Human APO4-α (hAPO4-α) is encoded by a cDNA
sequence related to IMAGE consortium clone 340844, which
was identified by searching the EST database (dbEST) for
sequence homology to the cytoplasmic tail of full-length
mAPO4-α protein using the TBLASTN algorithm. The

15 full-length nucleotide sequence encoding hAPO4-α is shown
in Figure 7A. This hAPO4-α nucleotide sequence (Figure
7C) encodes a hAPO4-α polypeptide of 423 amino acids
having 68.4% amino acid identity and 79.2% amino acid
sequence similarity with mAPO4-α.

20   The full-length hAPO4-α sequence was obtained
using 5' RACE and human fetal spleen Marathon-ready cDNA
(Clontech) according to the manufacturer's instructions.
Primer SEQ ID NO:49 (5'-TAGCTGGCTTCTCATAGTTAGTGC-3') and
AP1 primer SEQ ID NO:34 were used for the first round of

25 PCR. Primer SEQ ID NO:50
(5'-CCTGAGTTGATGCTGATTCTACCA-3') and AP2 primer SEQ ID
NO:36 were used for a subsequent round of nested PCR.
Inserts from eight or more individual clones were PCR
amplified and sequenced essentially as described above.

Characterization of rat APO4-α

Rat APO4-α (mAPO4-α) is another APO4 polypeptide; the rAPO4-α cDNA is related to IMAGE consortium clone 319945 which was identified by searching 5 the EST database (dbEST) for sequences with homology to the cytoplasmic tail of mAPO4-α protein using the TBLASTN algorithm. The partial cDNA sequence, shown in Figure 7D, lacks the 5' region. The partial rAPO4-α nucleotide sequence shares 95% amino acid sequence identity and 10 about 81% nucleotide sequence identity with the cytoplasmic domain of mAPO4-α.

Characterization of murine APO4-β

Murine APO4-β (mAPO4-β) is another APO4 polypeptide; the mAPO4-β cDNA is related to IMAGE 15 consortium clone 835418, derived from a Knowles Solter mouse blastocyst library. The IMAGE consortium clone was identified as described above. The partial cDNA sequence, shown in Figure 8, represents the carboxy-terminal 54 residues of the polypeptide. The 20 encoded polypeptide fragment bears about 77% amino acid sequence identity and about 81% amino acid similarity with mAPO4-α. The mAPO4-β fragment also bears about 74% amino acid identity and about 80% amino acid similarity with rAPO4-α.

APO4 Expression in Adult and Embryonic Tissues

        Expression of APO4 in adult human tissues was
studied by northern analysis using a multiple tissue
northern blot obtained from Clontech (7759-1) following
5   the manufacturer's instructions. The protein coding
region of hAPO4 cDNA was radiolabeled with P^{32} and used as
a probe. High level APO4 expression was seen in the
prostate gland, with very low expression seen in other
tissues including spleen, thymus, testis, uterus, small
10 intestine, colon and peripheral blood leukocytes.

        The prostate gland consists primarily of
fibroblast-like stromal cells and glandular epithelial
cells, with the latter being the site of origin of
prostatic carcinoma. Reverse-transcriptase/polymerase
15 chain reaction (RT-PCR) was used to test the expression
of hAPO4 in a prostate carcinoma derived epithelial cell
line, LNCaP (ATCC, Bethesda, MD). Total RNA isolated
from LNCaP cells was used for the first strand cDNA
preparation using random hexamer primers and Superscript
20 II Reverse Transcriptase (Life Technologies). The RT-PCR
procedure was performed following the manufacturer's
instruction with forward primer SEQ ID NO:57
(5'-GTCATGTAAAGTGACTTGTG-3') and reverse primer SEQ ID
NO:58 (5'-GAGCCGTTGTACTGAATGTCCTG-3'). A specific band
25 corresponding to hAPO4 was detected in RNA from LNCaP
cells, indicating that APO4 is expressed in epithelial
derived prostate cancer cell lines. Essentially similar
results were obtained in another prostate cancer cell
line, P65.

30   APO4 expression during embryonic development
was assayed by northern analysis of a multiple tissue

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northern blot obtained from Clontech (7763-1), which
contained polyA RNA from day 7, 11, 15 and 17 mouse
embryos. The protein coding region of mAPO4 cDNA was
radiolabeled with P^{32} and used as a probe. A strong
5 signal was detected in samples containing RNA from day
11, 15 and 17 embryos, indicating that APO4 is expressed
during early embryonic development.

hAPO4 induces apoptosis

The ability of hAPO4 to induce apoptosis was
10 assayed following transient transfection into the human
embryonic kidney 293T cell line. Plasmids encoding
various inhibitors were cotransfected to test for their
ability to modulate hAPO4 mediated apoptosis in 293T
cells.

15   A mammalian expression construct encoding human
APO4 was constructed by amplifying the sequence encoding
amino acids 23 to 423 of hAPO4 with a 5' primer
containing a Bgl II site and a 3' primer containing a
stop codon followed by a Sal I site, as described
20 previously for the APO8 construct. Following digestion
of the PCR product with Bgl II and Sal I, the resulting
product was inserted into a modified pSecTag A vector as
described previously.

Expression of the APO4 polypeptide described
25 above was confirmed by transfecting the above construct
into 293T cells using calcium phosphate transfection.
After 24 hours, cell lysates were prepared, the proteins
resolved by SDS-PAGE, and MYC-tagged hAPO4 detected by
western blot analysis using rabbit polyclonal antibody
30 against the MYC epitope tag (Santa Cruz Biotechnology,
Inc., Santa Cruz, California) following the

manufacturer's instructions. A single band of
approximately 55 kDa was detected in cells transfected
with the MYC-APO4 construct.

5   Constructs encoding various inhibitor plasmids
were prepared as follows. A K13-ORF (GenBank U90534) was
prepared by PCR amplification of the desired coding
sequence from a human genomic DNA sample containing
KSHV/HHV8 (human herpesvirus 8) genomic DNA (obtained
from Dr. Tim Rose; University of Washington, Seattle,
10   WA). Primers for PCR amplification were based on the
published sequence of K13-ORF with additional 5' sequence
for subsequent restriction digestion and cloning of the
amplified insert. Constructs encoding p35 were prepared
by using a baculovirus vector pFastBac HTa (Life
15   Technologies, Inc.) as the template. DN-IκBα or IκBα-ΔN
(missing the N-terminal 36 amino acids) and IκBα-S32/36A
(Brockman et al., Mol. Cell. Biol., 15: 2809-18, 1995);
NFκB driven luciferase reporter construct (Berberch et
al., J. Immunol., 153:4357-66, 1994); an active site
20   mutant of full-length rat MEKK1 (MEKK1-D1369A) (Xu et
al., Proc. Natl. Acad. Sci. USA, 93:5291-5295) and the
JNK binding domain of JIP-1 have been described
previously (Dickens et al., Science 277:693-696, 1997).
DN-mTRAF2, which encodes a TRAF2 polypeptide lacking the
25   first 87 amino acids, was generated by amplifying the DNA
encoding amino acids 88 to 501 of mTRAF2 using the IMAGE
consortium EST clone 439083 as a template and
incorporating a start site (i.e. methionine residue) at
the N-terminus.

30   Cell transfection was performed using calcium
phosphate coprecipitation (Sambrook et al., supra, 1989).
Briefly, 293T cells (1.2×10^5 cells) were cotransfected
with 100 ng/well hAPO4 expression plasmid or control

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plasmid along with lacZ reporter plasmid and 750 ng/well
empty vector or one of various inhibitor plasmids (p35,
Caspase 8 C360S, dominant-negative FADD (DN-FADD),
MRITα1, K13-ORF, DN-IκBα, JNK binding domain (JBD) of
5   JIP-1, BCLxL or the p65 subunit of NF-κB) and lacZ
reporter plasmid and were assayed for
hAPO4-induced apoptosis. Thirty-six hours after
transfection, cells were fixed and stained with X-gal,
and the percentage apoptotic cells determined as
10   described previously.

     The results shown in Figure 14A indicate that
hAPO4 induces cell death and that the hAPO4-induced
apoptosis is activated independently of the FADD-Caspase
pathway. In particular, several inhibitors of the
15   FADD-Caspase pathway (caspase 8 C360S, DN-FADD, p35,
K13-ORF and MRITα1), when cotransfected with hAPO4,
failed to block APO4-mediated cell death. These results
demonstrate that, unlike a variety of receptors of the
TNF family, APO4 can mediate apoptosis independently of
20   the FADD-caspase pathway.

hAPO4 activates the Jun N-terminal kinase (JNK) pathway

     Activation of the JNK pathway by hAPO4 was
measured using the PathDetect c-Jun Trans-Reporting
system (Stratagene, La Jolla, CA). Briefly, 1.2 x 10^5
25   293T EBNA cells were cotransfected in duplicate with 250
ng/well of hAPO4 expression vector and 750 ng/well of
empty vector or inhibitor plasmid (JBD of JIP-1,
MEKK1-D1269A, or DN-TRAF2). A fusion-transactivator
plasmid containing the GAL4 DNA-binding domain fused to
30   the c-Jun transcription factor (pFA-cJun, 50 ng), a
reporter plasmid encoding the luciferase gene downstream
of the GAL4 Upstream Activating sequence (pFR-luc,

500 ng) and a β-galactosidase (pRSV/LacZ) reporter
construct (75 ng) were also included in the
cotransfection. Forty hours after transfection, cell
extracts were prepared and luciferase and β-galactosidase

5 activities measured. The luciferase activity was
normalized relative to the β-galactosidase activity to
control for differences in transfection efficiency.

        As shown in Figure 14B, expression of hAPO4
produced significant activation of c-jun. Furthermore,

10 cotransfection of hAPO4 with JBD-JIP, and to a lesser
extent with MEKK1-D1369A or DN-TRAF2, prevented
APO4-mediated activation of the JNK pathway. These
results indicate that APO4-indcued apoptosis can be
mediated through activation of the JNK pathway and MEKK1.

15 *hAPO4 induces NF-κB*

        To test the possibility that APO4 can regulate
NF-κB expression, hAPO4 expression constructs were
cotransfected into 293T cells with a NF-κB luciferase
reporter. Transfection and NF-κB activation experiments

20 were performed as described previously. Inhibitor
plasmids (DN-TRAF2 or IκBα-S32/36A) were also
cotransfected to determine whether inhibition of the
TRAF2 or JNK pathway affects NF-κB activation by hAPO4.
As shown in Figure 14C, hAPO4 induced NF-κB expression,

25 and this activation was not blocked by dominant-negative
TRAF2. Also as shown in Figure 14C, hAPO4 failed to
activate NF-κB in the presence of IκB-S32/36A, an IκB
mutant that resists degradation in response to activators
of NF-κB. These results demonstrate that APO4 can

30 function, at least in part, through activation of NF-κB.
Given the multiple roles of NF-κB and the high level of
APO4 expression in embryonic development, these results

indicate that APO4 can stimulate growth in certain
contexts, in addition to its pro-apoptotic role described
above.

Deletion analysis of APO4

5   To determine the domains of APO4 which are
responsible for JNK activation and apoptosis, deletion
analysis was carried out using the following constructs:
pSecTag-MYC-APO4 encoding amino acid 23 to 423; pCDNA3.1
HisA-AP04.CP encoding the cytoplasmic domain of APO4
10   (amino acids 194 to 423); pSecTag-MYC-APO4-C377 encoding
amino acids 23 to 377; pSecTag-MYC-APO4-C355 encoding
amino acids 23 to 355; pSecTag-MYC-APO4-C286 encoding
amino acids 23 to 286; pSecTag-MYC-APO4-C172 encoding the
extracellular domain (amino acids 23 to 172).

15   These constructs were prepared by PCR
amplification of the nucleic acid fragment encoding the
designated amino acids using custom primers with
appropriate restriction enzyme containing sites and by
using human APO4 cDNA as template. The desired PCR
20   fragment was subsequently cloned into the modified
pSecTag vector described previously or the pCDNA3.1 HisA
vector (Invitrogen).

          The constructs were subsequently tested for
25   their ability to activate the JNK pathway in 293EBNA
cells as described above. The results indicated that,
while the full-length APO4 and its cytoplasmic domain
were able to activate the JNK pthway, the construct
encoding the extracellular domain failed to do so.
30   Furthermore, the pSecTag MYC-APO4-C286 failed to
significantly activate the JNK pathway although the

pSecTag MYC-APO4-C377 and C355 constructs could do so.
could.

        The above constructs were also tested for their
   ability to induce cell death in the 293T cells using the
5 assay decribed before. While the full-length APO4
   polypeptide, its cytoplasmic domain, C377, and C355
   constructs were able to effectively induce cell death,
   the C286 and the C172 constructs were inactive. These
   results indicate that the C-terminal 68 amino acids are
10 not essential for the APO4-induced activation of the JNK
   pathway or apoptosis and that these activities reside
   among the amino acids 194 to 355.

                           EXAMPLE V
15   Identification and Characterization of APO6

        This example describes the identification and
   characterization of a nucleic acid sequence encoding
   human APO6.

        Human APO6 (hAPO6) is a cDNA related to IMAGE
20 consortium clones 366305, 592256, 343204 and 591875,
   which have some similarity with the extracellular domain
   of human TNFR-2. These clones were identified by
   searching the EST database (dbEST) for sequences with
   homology to the extracellular domain of human TNFR-2
25 protein using the TBLASTN algorithm. The partial
   nucleotide sequence, lacking both 5' and 3' sequence, is
   shown in Figure 9. The partial hAPO6 sequence shows
   significant homology to the extracellular domains of
   TNFR-2, TNFR-1, and other members of the TNFR family
30 (about 16-33% amino acid sequence identity and about 30-
   50% amino acid sequence similarity) (Figure 1 and Table

1). Based on this homology, hAPO6 is classified as a new
member of the TNFR family.

EXAMPLE VI
Identification and Characterization of Tumor Necrosis
5   Factor Related Ligands

        This example describes the identification and
characterization of nucleic acid sequences encoding human
and murine TNRL1-α and TNRL1-β. This example also
demonstrates that human TNRL1-α is a cytotoxic ligand.

10 *Characterization of human TNRL1-α*

        Human tumor necrosis factor related ligand 1-α
(hTNRL1-α) is encoded by a cDNA clone related to IMAGE
consortium clones 593690, 115371 and 129696, which are
clones with homology to human TNF-α. The full length
15 cDNA encodes a predicted protein of 285 amino acids with
significant sequence homology to TNF-α as well as other
members of the TNF family (Figures 10 and 11A).

        The 5' end of the human TNRL-α sequence was
20 obtained using 5' RACE on Marathon Ready cDNA derived
from human leukocytes (Clontech) as described previously.
The first round of RACE was performed using AP1 primer
SEQ ID NO:34 as the forward primer and SEQ ID NO:59 (5'-
GTTGGTGTTTCACTGTCTGCAATCAG-3') as the reverse primer.
25 The race products were diluted 1:50 in water and used for
a second round of RACE using forward primer SEQ ID NO:36
and SEQ ID NO:60 (5'-GTTCTGACTGGAGTTGCCTTCTCCTG-3')
reverse primer. As in other members of the TNRL family,
the 5' sequence encodes a membrane-anchoring domain.
30 This factor, hTNRL1-α, is designated a new member of the

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TNF family which can play an important role in the
modulation of the immune and nervous systems.

Human TNRL1-α is a cytotoxic ligand

5   His-FLAG epitope-tagged extracellular portions
of hTNRL1-α, hTNRL3 or cytotoxic TRAIL ligand were
produced in bacteria. Western analysis of bacterial
lysates with an anti-FLAG antibody demonstrated that
hTNRL1-α, hTNRL3 and TRAIL extracellular polypeptide
portions were produced. BJAB cells were treated with
10   ligand, and cell survival measured after twenty-four
hours using the MTT assay in wells pre-coated with
anti-FLAG monoclonal antibody. The relative survival of
BJAB cells treated with hTNRL1-α, hTNRL3 or TRAIL was
significantly reduced as compared to control cells.
15   These results indicate that hTNRL1-α, like TRAIL, is a
cytotoxic ligand.

       Epitope tagged hTNRL1-α, hTNRL3 and TRAIL
polypeptide fragments were prepared in a modified
pET28(b) vector (Novagen Madison, WI), which has a FLAG
20   epitope downstream of the His epitope, and the sequences
confirmed by automated fluorescent sequencing. The
His-FLAG-tagged polypeptide fragments were expressed in
bacteria and purified by nickel-affinity chromatography
(Qiagen) according to the manufacturer's instructions.
25

       For cell survival assays, a 96-well plate was
pre-treated by incubation with 100 µl/well 10 µg/µl
anti-FLAG monoclonal antibody for two hours at 37°C.
BJAB cells were plated at 2 x 10⁴ cells/well and treated
30   with 5 µl purified ligand. Cell survival was measured
after 24 hours using the MTT assay (SIGMA) according to
the manufacturer's instructions.

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Characterization of murine TNRL1-α

Murine tumor necrosis factor related ligand 1-α (mTNRL1-α) is encoded by a cDNA related to IMAGE consortium clones 722549, 803594 and 791670. These
5   clones were identified by searching the dbEST database (NCBI) for sequences with homology to hTNRL1-α polypeptide (SEQ ID NO:20) using the TBLASTN algorithm. Murine TNRL1-α sequence was obtained using 5' RACE on murine spleen Marathon-ready cDNA (Clontech) according to
10 the manufacturer's instructions. Primer SEQ ID NO:51 (5'-GTCTCCGTTGCGTGAAATCTGTGC-3') and AP1 primer SEQ ID NO:34 were used for the first round of PCR. Primer SEQ ID NO:52 (5'-AGGAATTGTTGGGCAGTGTTTTGG-3') and AP2 primer SEQ ID NO:36 were used for a subsequent round of nested
15 PCR. The RACE products were cloned into vector PCR2.1 using the TA cloning kit (Invitrogen) and sequenced on an automated fluorescent sequencer (ABI 373) essentially as described before.

The available mTNRL1-α sequence is shown in
20 Figure 11B. The predicted mTNRL1-α sequence is highly homologous to hTNRL1-α at both the nucleotide and protein levels and indicates a type II membrane protein with a putative N-terminal cytoplasmic domain (amino acids 1-46, a transmembrane domain (amino acids 47-74) and a large
25 extracellular receptor binding domain (amino acids 75-290).

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Characterization of human TNRL1-β

Human tumor necrosis factor related ligand 1-β
(hTNRL1-β) is encoded by a cDNA related to IMAGE
consortium clones 306307, 489038 and 306064, identified
5   as described above, and which exhibit homology to
TNRL1-α. The hTNRL1-β sequence, shown in Figure 12A,
encodes a predicted polypeptide of 250 amino acids with
sequence homology to TNRL1-α and TNF-α as well as other
members of TNF family (see Figure 10). The sequence
10   indicates a type II membrane protein with a putative N-
terminal cytoplasmic domain (amino acids 1-25), a
transmembrane hydrophobic domain (26-50) and a large
extracellular receptor binding domain (amino acids 51-
250).

15   Characterization of murine TNRL1-β

Murine tumor necrosis factor related ligand 1-β
(mTNRL1-β) is encoded by a cDNA related to IMAGE
consortium clone 876879. This clone was identified by
searching the dbEST database (NCBI) for sequences with
20   homology to hTNRL-β polypeptide (SEQ ID NO:24) using the
TBLASTN algorithm. The full-length mTNRL1-β cDNA
sequence is shown in Figure 12B. The sequence indicates
a type II membrane protein with a putative N-terminal
cytoplasmic domain (amino acids 1-16), a transmembrane
25   hydrophobic domain (amino acids 17-42) and a large
extracellular receptor binding domain (amino acids
43-241).

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EXAMPLE VII

Identification and Characterization of TNRL3

This example describes the identification and
characterization of nucleic acid sequences encoding human

5   and murine TNRL3. This example also demonstrates that
human TNRL3 is a cytotoxic ligand.

Characterization of human TNRL3

Human tumor necrosis factor related ligand 3
(hTNRL3) is related to IMAGE consortium clones 154742 and

10   271670 and Genbank clone C00994. These sequences were
identified by searching the dbEST database (NCBI) for
sequences with homology to human TRAIL ligand protein
using the TBLASTN olgorithym. 5' RACE with human fetal
spleen marathon ready cDNA (Clontech) was performed

15   according to the manufacturer's instructions to obtain
additional 5' sequence. Primer SEQ ID NO:53
(5'-CGAAGTAGGTGAGGAAGGGGGCAG-3') and AP1 primer SEQ ID
NO:34 were used for the first round of PCR. Primer SEQ
ID NO:54 (5'-GTAGACAGCCTTCCCCTCATCAAA-3') and AP2 primer

20   SEQ ID NO:36 were used for a subsequent round of nested
PCR.

The partial hTNRL3 sequence shown in Figure 13A
lacks sequence at the 5' end of the molecule and encodes
the ligand-binding extracellular domain. The missing 5'

25   sequence can encode a membrane anchoring sequence. The
partial sequence shown in Figure 13A exhibits homology to
TRAIL (TNF Related Apoptosis Inducing Ligand) and other
members of the TNF family (see Figure 10).

Human TNRL3 is a cytotoxic ligand

        BJAB cells were treated with epitope-tagged
extracellular portions of hTNRL1-α, hTNRL3 or TRAIL, and
cell survival measured after twenty-four hours using the
5   MTT assay as described above. The relative survival of
BJAB cells treated with epitope-tagged hTNRL1-α, hTNRL3
or TRAIL extracellular fragments was significantly
reduced as compared to control cells. These results
indicate that hTNRL3 functions as a cytotoxic ligand.

10   *Characterization of murine TNRL3*

        Murine tumor necrosis factor related ligand 3
(TNRL3) is encoded by a cDNA related to IMAGE consortium
clone 696209. This clone was identified by searching the
dbEST database for sequences with homology to hTNRL3
15   protein (SEQ ID NO:28) using the TBLASTN algorithm. The
available sequence of clone 696209 is incomplete at the
5' end. Additional murine TNRL3 sequence was obtained
using 5' RACE on murine spleen Marathon-ready cDNA
(Clontech) according to the manufacturer's instructions.
20   Primer SEQ ID NO:55 (5'-CACACCGTTCACCAGCAAGTCCAG-3') and
AP1 primer SEQ ID NO:34 were used for the first round of
PCR. Primer SEQ ID NO:56
(5'-GGTAGACAGCCTTTCCCTCATCAA-3') and AP2 primer SEQ ID
NO:36 were used for a subsequent round of nested PCR.
25   The RACE products were cloned into vector PCR2.1 using
the TA cloning kit (Invitrogen) and sequenced on an
automated fluorescent sequencer (ABI 373).

        The partial mTNRL3 sequence, lacking the 5'
end, is shown in Figure 13B. The mTNRL3 cDNA encodes a
30   polypeptide with significant sequence homology to hTNRL3.

All journal article, reference, and patent citations provided above, in parentheses or otherwise, whether previously stated or not, are incorporated herein by reference.

5   Although the invention has been described with reference to the disclosed embodiments, those skilled in the art will readily appreciate that the specific experiments detailed are only illustrative of the invention. It should be understood that various
10   modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

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What is claimed is:

1.   An isolated APO4 polypeptide, comprising
substantially the same amino acid sequence as an APO4
polypeptide, or an active fragment thereof.

5   2.   The isolated polypeptide of claim 1,
comprising substantially the same amino acid sequence as
an amino acid sequence selected from the group consisting
of mAPO4-αL (SEQ ID NO:8) or an active fragment thereof,
mAPO4-αS (SEQ ID NO:10) or an active fragment thereof,
10   hAPO4-α (SEQ ID NO:12) or an active fragment thereof, and
rAPO4-α (SEQ ID NO:14) or an active fragment thereof.

3.   The isolated polypeptide of claim 1,
comprising substantially the same amino acid sequence as
mAPO4-β (SEQ ID NO:16).

15   4.   The isolated polypeptide of claim 1,
wherein said active fragment comprises a soluble form of
an APO4 polypeptide having an APO4 polypeptide ligand
binding domain.

5.   An isolated nucleic acid molecule,
20   comprising a nucleotide sequence encoding substantially
the APO4 polypeptide of claim 1, or an active segment
thereof.

6.   An APO4 selective binding agent, said
agent comprising the activity of selectively binding to
25   the APO4 polypeptide of claim 1.

7.   A method of diagnosing prostate cancer in an individual, comprising determining the level of APO4 in said individual.

8.   A method of treating prostate cancer,
5 comprising administering to an individual having prostate cancer a conjugate comprising an APO4 selective binding agent linked to a therapeutic moiety.

9.   A method of identifying an effective pharmacological agent useful in the diagnosis or
10 treatment of a disease associated with APO4 activity, comprising the steps of:

a)   contacting an APO4 polypeptide or active fragment thereof with an agent, and

b)   determining selective binding of said
15   agent to said APO4 polypeptide or active fragment thereof,

wherein said selective binding indicates that said agent is an effective pharmacological agent useful in the diagnosis or treatment of a disease associated
20 with APO4 activity.

10.   The method of claim 9, wherein said APO4 polypeptide or active fragment thereof is an APO4 extracellular ligand binding domain.

11.   The method of claim 9, wherein said APO4
25 polypeptide or active fragment thereof is expressed in a cell or on the surface of a cell.

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12.   The method of claim 9, wherein said APO4 polypeptide or active fragment thereof is contacted with an agent *in vitro*.

13.   A method of screening for an APO4 agonist
5   useful in treating prostate cancer, comprising the steps of:

a)   contacting a cell expressing an APO4 polypeptide or active fragment thereof with an agent;

10   b)   assaying for increased APO4 activity,

wherein increased APO4 activity indicates that said agent is an APO4 agonist useful in treating prostate cancer.

14.   A method of screening for an APO4
15   antagonist, comprising the steps of:

a)   contacting a cell expressing an APO4 polypeptide or active fragment thereof with an agent;

b)   assaying for decreased APO4 activity,

20   wherein decreased APO4 activity indicates that said agent is an APO4 antagonist.

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15.   A method of identifying an effective
pharmacological agent useful in the diagnosis or
treatment of a disease associated with APO4 activity,
comprising the steps of:

5   a)   forming a mixture comprising an APO4
                 polypeptide or active fragment thereof, an
                 APO4 signal transducer molecule that
                 specifically interacts with a cytoplasmic
                 domain of said APO4 polypeptide or active
10   fragment thereof, and an agent;

           b)   detecting the level of APO4 activity
                 in the presence of said agent,

wherein an alteration in said APO4 activity relative to
control activity indicates that said agent is an
15 effective pharmacological agent useful in the diagnosis
or treatment of a disease associated with APO4 activity.

      16.   A method of claim 15, wherein said APO4
activity is measured *in vivo*.

      17.   A method of claim 15, wherein said APO4
20 activity is measured *in vitro*.

      18.   An isolated APO8 polypeptide, comprising
substantially the same amino acid sequence as APO8, or an
active fragment thereof.

      19.   The isolated polypeptide of claim 18,
25 comprising substantially the same amino acid sequence as
hAPO8 (SEQ ID NO:2), or an active fragment thereof.

20.   The isolated polypeptide of claim 18,
wherein said active fragment comprises a soluble form of
APO8 having an APO8 ligand binding domain.

21.   An isolated nucleic acid molecule,
5   comprising a nucleotide sequence encoding substantially
the APO8 polypeptide of claim 18, or an active segment
thereof.

22.   An APO8 selective binding agent, said
agent comprising the activity of selectively binding to
10   the APO8 polypeptide of claim 18.

23.   An isolated APO9 polypeptide, comprising
substantially the same amino acid sequence as APO9, or an
active fragment thereof.

24.   The isolated polypeptide of claim 23,
15   comprising substantially the same amino acid sequence as
hAPO9 (SEQ ID NO:6), or an active fragment thereof.

25.   The isolated polypeptide of claim 23,
wherein said active fragment comprises a soluble form of
APO9 having an APO9 ligand binding domain.

20   26.   An isolated nucleic acid molecule,
comprising a nucleotide sequence encoding substantially
the APO9 polypeptide of claim 23, or an active segment
thereof.

27.   An APO9 selective binding agent, said
25   agent comprising the activity of selectively binding to
the APO9 polypeptide of claim 23.

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28. An isolated APO6 polypeptide, comprising substantially the same amino acid sequence as APO6, or an active fragment thereof.

29. The isolated APO6 polypeptide of claim 28,
5   comprising substantially the same amino acid sequence as hAPO6 (SEQ ID NO:18), or an active fragment thereof.

30. The isolated polypeptide of claim 28, wherein said active fragment comprises a soluble form of APO6 having an APO6 ligand binding domain.

10   31. An isolated nucleic acid molecule, comprising a nucleotide sequence encoding substantially the APO6 polypeptide of claim 28, or an active segment thereof.

32. An APO6 selective binding agent, said
15 agent comprising the activity of selectively binding to the APO6 polypeptide of claim 28.

33. An isolated tumor necrosis factor related ligand 1 (TNRL1) polypeptide, comprising substantially the same amino acid sequence as TNRL1, or an active
20 fragment thereof.

34. The isolated TNRL1 polypeptide of claim 33, comprising substantially the same amino acid sequence as an amino acid sequence selected from the group consisting of hTNRL1-α (SEQ ID NO:20), or an active
25 fragment thereof, and mTNRL1-α (SEQ ID NO:22), or an active fragment thereof.

35. The isolated TNRL1 polypeptide of
claim 33, comprising substantially the same amino acid
sequence as an amino acid sequence selected from the
group consisting of hTNRL1-β (SEQ ID NO:24), or an active
5 fragment thereof, and mTNRL1-β (SEQ ID NO:26), or an
active fragment thereof.

36. The isolated polypeptide of claim 33,
wherein said active fragment comprises a soluble form of
TNRL1 having a TNRL1 receptor binding domain.
10

37. An isolated nucleic acid molecule,
comprising a nucleotide sequence encoding substantially
the TNRL1 polypeptide of claim 33, or an active segment
thereof.

15   38. A TNRL1 selective binding agent, said
agent comprising the activity of selectively binding to
the TNRL1 polypeptide of claim 33.

39. An isolated TNRL3 polypeptide, comprising
substantially the same amino acid sequence as TNRL3, or
20 an active fragment thereof.

40. The isolated TNRL3 polypeptide of
claim 39, comprising substantially the same amino acid
sequence as an amino acid sequence selected from the
group consisting of hTNRL3 (SEQ ID NO:28), or an active
25 fragment thereof, and mTNRL3 (SEQ ID NO:30), or an active
fragment thereof.

41. The isolated polypeptide of claim 39,
wherein said active fragment comprises a soluble form of
TNRL3 having a TNRL3 receptor binding domain.

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42.   An isolated nucleic acid molecule,
comprising a nucleotide sequence encoding substantially
the TNRL3 polypeptide of claim 39, or an active segment
5   thereof.

43.   A TNRL3 selective binding agent, said
agent comprising the activity of selectively binding to
the TNRL3 polypeptide of claim 39.

```
          1                                                              80
hAP04-α   ..........  ..........  ..........  .........   ..malkVLle  qektfftlLV  LlgylsckVT  cesgdcrqqe
hAP06     MeQrGqnApA  asGarkrhgP  GPReArgArp  ..........  VV..aAVLLi  VsAeSAiitq  qdLapqQrVA  PqQkRsSpse
hAP08     MgQnGpSArA  raG....raP  GPRdAReAsp  ..........  VV.vgVLLq   VvpsSAAtik  Lh..dQsig   tqQwehSpLg
APO8RP    ..........  ..........  ..........  ..........  VVVivAVLLd  VlAYSAttar  qeevpqqtVA  PqQqRhSFkg
hAP09     ..........  ..........  ..........  .......mi   GiWtlipLvl  .mvRiplqcV  LwgcLitaVh  PepPtacrek
CD40      ..........  ..........  ..........  ..........  tsVarissks  VnAqvtdins  kgLeLRktVt  tvetqn..Le
Fas       ..........  ..........  ..........  ........m   GLstvpdLiL  .meqrprG    caAvaAAlLi  trsPRcdCag
DR3       ..........  ..........  ..........  ..........  piVilelLVG  iypsgviglV  phLgdRekrd  svcPqgkY
TNFR1     ..........  ..........  ..........  .......Map  VaVwaAlavG  ielWaAAhal  paqvaftpYA  PepgstcrLr
TNFR2

          81                                                             160
hAP04-α   frdrsgncvp  skECgfgygE  rFkeDwgFqk  ....CkpCl   dCavvnRfqk  ancsaTsdai
hAP06     CnqCgPGmeL  DaqCvaCrih  ..........  ..ercRYc    nvlcgeReeE  araChaThNr
hAP08     gL........  .......SE   Dgr..dCisC  ndLlfCLRCT  rCDSg..evE  lSpCTTTRNT
APO8RP    EL........  .......SE   rpG..acnrc  nnLfaCLpCT  aCKsd..eeE  rSpCTTTRNT
hAP09     Ee........  .......SE   hTG..acnpC  nnepsCFpCT  vCKsd..gkh  kSsCImRdT
CD40      qYiinsq..C  CSilcqPGqkL eFldtWnr..  ethchqhkYc  dpnlgLRvqq  .kgTsetdT
Fas       gLH.hdgQfC  hkpCPPGerk  qeGkeYTdka  hfsSkCrRCr  lCDeqhglev  einCTrTqNT
DR3       DFHkkiglfC  CrgCPaghyL  pqdtflaWen  hhnSeCaRCq  aCDeqasqva  lenCsavadT
TNFR1     .iHpQnnslC  CtKChkGtyL  esGsftasen  hl.rhCLsCs  kCrkeMqqvE  lSsCIVdRdT
TNFR2     EYydQtaQmc  CSKCsPGqHa  TYtqlWnWvp  ecLSCgsRCs  sdqve...tq  .aCTreqNr
```

FIG. IA

```
                                   161                                                                        240
hAP04-α   cgdCIPGFYR  ktklvgfqdm  eCvpCgdpPP  pYe,,phCas  kvnlvklast  assprdtalA  AvlCsa....  ..........
hAP06     acrCrtgFFa  h,.....ag   fCLehasCPP  GagVlapgIP  sqntaCqPsp  pGtFSasSSs  sEqCqPhrNC  TalgLalnvp
hAP08     VCQCeegtFR  e,edSpEMCR  K..CrtgCpr  GmvKVygdCTP wSDleCVhkE  sG......    SGnghN....  ..........
AP08RP    aCQCKPGtFR  n.dnSaEMCR  K..CstgCPr  GmvKVkdCTP  wSDleCVhkE  sGnghN.,,,  ..........  ..........
hAP09     VCQCKeGtFR  n.enSPEMCR  K.,CsR.CPs  GevqVsnCTs  wdDIqCVeef  GanarvetpA  AEetmntSpg  TpapaaeEtm
CD40      ICtCeeGWhc  t...SEa.Ce  sCvihRsCsP  GFgVkqlaTg  vSDtiCePcd  vGfFSNvsSA  fEKChPwtsC  etkdLvvqaa
Fas       kCrcKPnFFq  nstvcE....  ..hCdpCtk   cehglikect  ltsntkckeE  Gsrs......  ..........  ..........
DR3       rCgcKPKPGWFv ecqvSqcvss spFYCqpCld  cg,alrrhlr  llCsrrdtdc  GtcLpgfyeh  gDgCVscpts  Tigscperca
TNFR1     VCgCrknqYR  hy,.:..WS   enLF.qCfn   lScqekqntv  ctchagffir  eneCVscSNC  kks...lEct
TNFR2     ICtCrPGWYc  alskqEg.CR  lCapIRKCrP  GFgVarpgTe  tSDvVCKPca  pGtFSNttSs  tDiCrPhqlC  n....vvalp

          241                                                                         309
hAP04-α   GssShDtiCT  sctgfPlStr  vPGAeecEra  Vidfvafqdi  sikrLSgccS  prgpegwGPt  PrAaarpss
hAP06     ..........  ..........  ..........  ..........  ..........  ..........  .........
hAP08     nTspgtpapa  aeETmttSpg  tPapaaeEtm  ttspgtpapa  aeEtMtTsPg  TpasshYlsc  t........
AP08RP    GTnktDvVCg  pqD..rl...  ..........  ..........  ..........  ..........  .........
hAP09     avcgWrQmfw  v.........  ..........  ..........  ..........  ..........  .........
CD40      klc,lpQlen  vkgTedsg,.  ..........  ..........  ..........  ..........  .........
Fas       ..........  ..........  ..........  ..........  ..........  ..........  .........
DR3       ..........  ..........  ..........  ..........  ..........  ..........  .........
TNFR1     ..........  ..........  ..........  ..........  ..........  ..........  .........
TNFR2     GnaSRDaVCT  sts..Ptrsm  aPGAvhlpqp  Vstrsqhtqp  tpEp.STaPS  Tsfllpm6Ps  PpAegstg,
```

FIG. IB

```
   1  CGGAGAACCCCGCAATCTCTGCGCCCACAAAATACACCGACGATGCCCGATCTACTTTAA    60
  61  GGGCTGAAACCCACGGGCCTGAGAGACTATAAGAGCCGTTCCCTACCGCCATGGAACAACG   120
                                                        M  E  Q  R    4
 121  GGGACAGAACGCCCCGGCCGCTTCGGGGGGCCCGGAAAAAGGCACGGCCCAGGACCCAGGA   180
   5   G  Q  N  A  P  A  A  S  G  A  R  K  R  H  G  P  G  P  R  E    24
 181  GGCGCGGGGGAGCCAGGCCTGGGCTCCGGGTCCCCAAGACCCTTGTGCTCGTTGTCGCCGC   240
  25   A  R  G  A  R  P  G  L  R  V  P  K  T  L  V  L  V  V  A  A    44
 241  GGTCCTGCTCGTTGGTCTCAGCTGAGCTCGCTGTCTGATCACCCAACAAGACCTAGCTCCCCA  300
  45   V  L  L  L  V  S  A  E  S  A  L  I  T  Q  Q  Q  D  L  A  P  Q   64
 301  GCAGAGAGTGGCCCCACAACAAAAGAGGTCCAGCCCCTCAGAGGGATTGTGTCCACCTGG   360
  65   Q  R  V  A  P  Q  Q  K  R  S  S  P  S  E  G  L  C  P  P  G    84
 361  ACACCATATCTCAGAAGACGGTAGAGATTGCATCTCCTGCAAATATGGACAGGACTATAG   420
  85   H  H  I  S  E  D  G  R  D  C  I  S  C  K  Y  G  Q  D  Y  S   104
 421  CACTCACTGGAATGACCTCCTTTTCTGCTTGCGCTGCACCAGGTGTGATTCAGGTGAAGT   480
 105   T  H  W  N  D  L  F  C  L  R  C  T  R  C  D  S  G  E  V    124
 481  GGAGCTAAGTCCCTGCACCACGACCAGAAACACAGTGTGTCAGTGCGAAGAAGGCACCTT   540
 125   E  L  S  P  C  T  T  T  R  N  T  V  C  Q  C  E  E  G  T  F   144
 541  CCGGGAAGAAGATTCTCCTGAGATGTGCCGGAAGTGCCGCACAGGGTGTCCCAGAGGGAT   600
 145   R  E  E  D  S  P  E  M  C  R  K  C  R  T  G  C  P  R  G  M   164
 601  GGTCAAGGTCGGTGATTGTACACCCTGGAGTGACATCGAATGTGTCCACAAAGAGTCAGG   660
 165   V  K  V  G  D  C  T  P  W  S  D  I  E  C  V  H  K  E  S  G   184
 661  CATCATCATAGGAGTCACAGTTGCAGCCGTAGTCTTGATTGTGGCTGTGTTTGTTTGCAA   720
 185   I  I  I  G  V  T  V  A  A  V  V  L  I  V  A  V  F  V  C  K   204
 721  GTCTTTACTGTGGAAGAAAGTCCTTCCTTACCTGAAAGGCATCTGCTCAGGTGGTGGTGG   780
 205   S  L  L  W  K  K  V  L  P  Y  L  K  G  I  C  S  G  G  G  G   224
 781  GGACCCTGAGCGTGTGGACAGAAGCTCACAACGACCTGGGGCTGAGGACAATGTCCTCAA   840
 225   D  P  E  R  V  D  R  S  S  Q  R  P  G  A  E  D  N  V  L  N   244
 841  TGAGATCGTGAGTATCTTGCAGCCCACCCAGGTCCCTGAGCAGGAAATGGAAGTCCAGGA   900
 245   E  I  V  S  I  L  Q  P  T  Q  V  P  E  Q  E  M  E  V  Q  E   264
 901  GCCAGCAGAGCCAACAGGTGTCAACATGTTGTCCCCCGGGGAGTCAGAGCATCTGCTGGA   960
 265   P  A  E  P  T  G  V  N  M  L  S  P  G  E  S  E  H  L  L  E   284
 961  ACCGGCAGAAGCTGAAAGGTCTCAGAGGAGGAGGCTGCTGGTTCCAGCAAATGAAGGTGA  1020
 285   P  A  E  A  E  R  S  Q  R  R  R  L  L  V  P  A  N  E  G  D   304
1021  TCCCACTGAGACTCTGAGACAGTGCTTCGATGACTTTGCAGACTTGGTGCCCTTTGACTC  1080
 305   P  T  E  T  L  R  Q  C  F  D  D  F  A  D  L  V  P  F  D  S   324
1081  CTGGGAGCCGCTCATGAGGAAGTTGGGCCTCATGGACAATGAGATAAAGGTGGCTAAAGC  1140
 325   W  E  P  L  M  R  K  L  G  L  M  D  N  E  I  K  V  A  K  A   344
1141  TGAGGCAGCGGGCCACAGGGACACCTTGTACACGATGCTGATAAAGTGGGTCAACAAAAC  1200
 345   E  A  A  G  H  R  D  T  L  Y  T  M  L  I  K  W  V  N  K  T   364
1201  CGGGCGAGATGCCTCTGTCCACACCCTGCTGGATGCCTTGGAGACGACTCTGGGAGAGACT  1260
 365   G  R  D  A  S  V  H  T  L  L  D  A  L  E  T  L  G  G  E  R  L  384
1261  TGCCAAGCAGAAGATTGAGGACCACTTGTTGAGCTCTGGAAAGTTCATGTATCTAGAAGG  1320
 385   A  K  Q  K  I  E  D  H  L  L  S  S  G  K  F  M  Y  L  E  G   404
1321  TAATGCAGACTCTGCCATGTCCTAAGTGTGATTCTCTTCAGGAAGTCAGACCTTCCCTGG  1380
 405   N  A  D  S  A  M  S  •                                      424
1381  TTTACCTTTTTTCTGGAAAAAGCCCAACTGGACTCCAGTCAGTAGGAAAGTGCCACAATT  1440
1441  GTCACATGACCGGTACTGGAAGAAACTCTCCCATCCAACATCACCCAGTGGATGGAACAT  1500
1501  CCTGTAACTTTTCACTGCACTTGGCATTATTTTTATAAGCTGAATGTGATAATAAGGACA  1560
1561  CTATGGAAATGTCTGGATCATTCCGTTTGTGCGTACTTTGAGATTTGGTTTGGGATGTCA  1620
1621  TTGTTTTCACAGCACTTTTTTATCCTAATGTAAATGCTTTATTTATTTATTTGGGGTACA  1680
1681  TTGTAAGATCCATCTACACAGTCGTAAAAAAAAAAA                         1717
```

FIG. 2

```
   1  ATGGCGCCACCACCAGCTAGAGTACATCTAGGTGCGTTCCTGGCAGTGACTCCGAATCCC   60
  61  GGGAGCGCAGCGAGTGGGACAGAGGCAGCCGCGGCCACACCCAGCAAAGTGTGGGGCTCT  120
 121  TCCGCGGGGAGGATTGAACCACGAGGCGGGGGCCGAGGAGCGCTCCCTACCTCCATGGGA  180
 -17                                                           M  G     2
 181  CAGCACGGACCCAGTGCCCGGGCCCGGGCAGGGCGCGCCCCAGGACCCAGGCCGGCGCGG  240
   3   Q  H  G  P  S  A  R  A  R  A  R  A  G  R  A  P  G  P  R  P  A  R   22
 241  GAAGCCAGCCCTCGGCTCCGGGTCCACAAGACCTTCAAGTTTGTCGTCGTCGGGGTCCTG  300
  23   E  A  S  P  R  L  R  V  H  K  T  F  K  F  V  V  V  G  V  L   42
 301  CTGCAGGTCGTACCTAGCTCAGCTGCAACCATCAAACTTCATGATCAATCAATTGGCACA  360
  43   L  Q  V  V  P  S  S  A  A  T  I  K  L  H  D  Q  S  I  G  T   62
 361  CAGCAATGGGAACATAGCCCTTTGGGAGAGTTGTGTCCACCAGGATCTCATAGATCAGAA  420
  63   Q  Q  W  E  H  S  P  L  G  E  L  C  P  P  G  S  H  R  S  E   82
 421  CGTCCTGGAGCCTGTAACCGGTGCACAGAGGGTGTGGGTTACACCAATGCTTCCAACAAT  480
  83   R  P  G  A  C  N  R  C  T  E  G  V  G  Y  T  N  A  S  N  N   102
 481  TTGTTTGCTTGCCTCCCATGTACAGCTTGTAAATCAGATGAAGAAGAGAGAAGTCCCTGC  540
 103   L  F  A  C  L  P  C  T  A  C  K  S  D  E  E  E  R  S  P  C   122
 541  ACCACGACCAGGAACACAGCATGTCAGTGCAAACCAGGAACTTTCCGGAATGACAATTCT  600
 123   T  T  T  R  N  T  A  C  Q  C  K  P  G  T  F  R  N  D  N  S   142
 601  GCTGAGATGTGCCGGAAGTGCAGCACAGGTGCCCCAGAGGGATGGTCAAGGTCAAGGAT  660
 143   A  E  M  C  R  K  C  S  T  G  C  P  R  G  M  V  K  V  K  D   162
 661  TGTACGCCCTGGAGTGACATCGAGTGTGTCCACAAAGAATCAGGCAATGGACATAATATA  720
 163   C  T  P  W  S  D  I  E  C  V  H  K  E  S  G  N  G  H  N  I   182
 721  TGGGTGATTTTGGTTGTGACTTTGGTTGTTCCGTTGCTGTTGGTGGCTGTGCTGATTGTC  780
 183   W  V  I  L  V  V  T  L  V  V  P  L  L  L  V  A  V  L  I  V   202
 781  TGTTGTTGCATCGGCTCAGGTTGTGGAGGGGACCCCAAGTGCATGGACAGGGTGTGTTTC  840
 203   C  C  C  I  G  S  G  C  G  G  D  P  K  C  M  D  R  V  C  F   222
 841  TGGCGCTTGGGTCTCCTACGGGGCCTGGGGCTGAGGACAATGCTCACAACGAGATTCTG  900
 223   W  R  L  G  L  L  R  G  L  P  G  A  E  D  N  A  H  N  E  I  L   242
 901  AGCAACGCAGACTCGCTGTCCACTTTCGTCTCTGAGCAGCAAATGGAAAGCCAGGAGCCG  960
 243   S  N  A  D  S  L  S  T  F  V  S  E  Q  Q  M  E  S  Q  E  P   262
 961  GCAGATTTGACAGGTGTCACTGTACAGTCCCCAGGGGAGGCACAGTGTCTGCTGGGACCG 1020
 263   A  D  L  T  G  V  T  V  Q  S  P  G  E  A  Q  C  L  L  G  P   282
1021  GCAGAAGCTGAAGGGTCTCAGAGGAGGAGGCTGCTGGTTCCAGCAAATGGTGCTGACCCC 1080
 283   A  E  A  E  G  S  Q  R  R  R  L  L  V  P  A  N  G  A  D  P   302
1081  ACTGAGACTCTGATGCTGTTCTTTGACAAGTTTGCAAACATCGTGCCCTTTGACTCCTGG 1140
 303   T  E  T  L  M  L  F  F  D  K  F  A  N  I  V  P  F  D  S  W   322
1141  GACCAGCTCATGAGGCAGCTGGACCTCACGAAAAATGAGATCGATGTGGTCAGAGCTGGT 1200
 323   D  Q  L  M  R  Q  L  D  L  T  K  N  E  I  D  V  V  R  A  G   342
1201  ACAGCAGGCCCAGGGGATGCCTTGTATGCAATGCTGATGAAATGGGTCAACAAAACTGGA 1260
 343   T  A  G  P  G  D  A  L  Y  A  M  L  M  K  W  V  N  K  T  G   362
1261  CGGAACGCCTCGATCCACACCCTGCTGGATGCCTTGGAGAGGATGGAAGAGAGACATGCA 1320
 363   R  N  A  S  I  H  T  L  L  D  A  L  E  R  M  E  E  R  H  A   382
1321  AAAGAGAAGATTCAGGACCTCTTGGTGGACTCTGGAAAGTTCATCTACTTAGAAGATGGC 1380
 383   K  E  K  I  Q  D  L  L  V  D  S  G  K  F  I  Y  L  E  D  G   402
1381  ACAGGCTCTGCCGTGTCCTTGGAGTGA                                  1407
 403   T  G  S  A  V  S  L  E  *                                   411
```
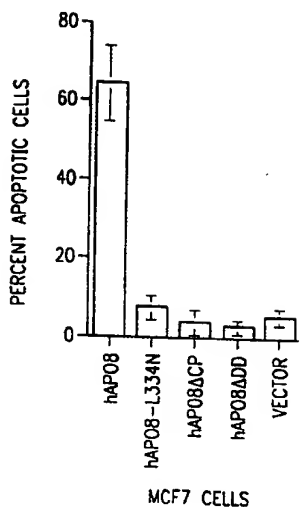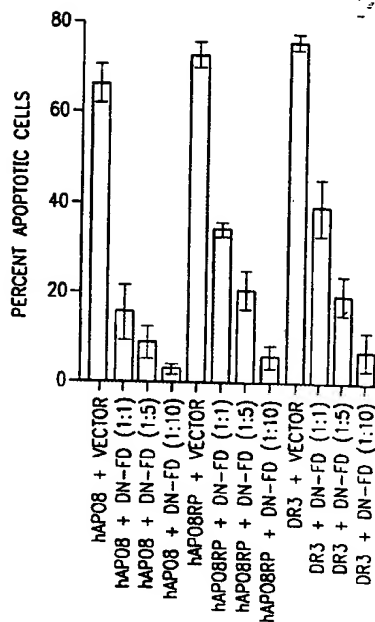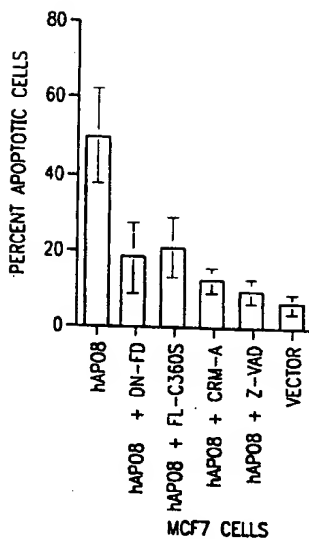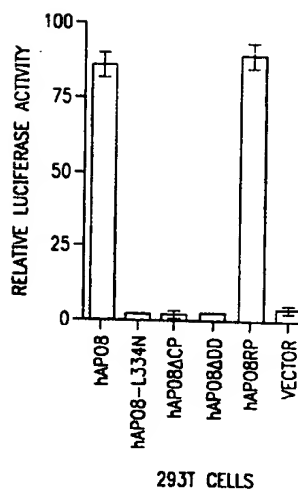
FIG. 3

FIG. 4A

FIG. 4B

FIG. 5A

FIG. 5B

FIG. 5C

FIG. 5D

```
   1 CACGCGCACGAACTCAGCCAACGATTTCTGATAGATTTTTGGGAGTTTGACCAGAGATGC   60
  61 AAGGGGTGAAGGAGCGCTTCTACCGTTAGGAACTCTGGGGACAGAGCGCCCCGGCCGCC  120
 121 TGATGGCCGAGGCAGGGTGCGACCCAGGACCCAGGACGGCGTCGGGAACCATACCATGGC  120
 -17                                                          M  A  2
 181 CCGGATCCCCAAGACCCTAAAGTTCGTCGTCGTCATCGTCGCGGTCCTGCTGCCAGTCCT  240
   3  R  I  P  K  T  L  K  F  V  V  V  V  I  V  A  V  L  L  P  V  L  22
 241 AGCTTACTCTGCCACCACTGCCCGGCAGGAGGAAGTTCCCCAGCAGACAGTGGCCCCACA  300
  23  A  Y  S  A  T  T  A  R  Q  E  E  V  P  Q  Q  T  V  A  P  Q  42
 301 GCAACAGAGGCACAGCTTCAAGGGGGGAGGAGTGTCCAGCAGGATCTCATAGATCAGAACA  360
  43  Q  Q  R  H  S  F  K  G  G  E  E  C  P  A  G  S  H  R  S  E  H  62
 361 TACTGGAGCCTGTAACCCGTGCACAGAGGGTGTGGATTACACCAACGCTTCCAACAATGA  420
  63  T  G  A  C  N  P  C  T  E  G  V  D  Y  T  N  A  S  N  N  E  82
 421 ACCTTCTTGCTTCCCATGTACAGTTTGTAAATCAGATCAAAAACATAAAGTTCCTGCAC  480
  83  P  S  C  F  P  C  T  V  C  K  S  D  Q  K  H  K  S  S  C  T  102
 481 CATGACCAGAGACACAGTGTGTCAGTGTAAAGAAGGCACCTTCCGGAATGAAAACTCCCC  540
 103  M  T  R  D  T  V  C  Q  C  K  E  G  T  F  R  N  E  N  S  P  122
 541 AGAGATGTGCCGGAAGTGTAGCAGGTGCCCTAGTGGGGAAGTCCAAGTCAGTAATTGTAC  600
 123  E  M  C  R  K  C  S  R  C  P  S  G  E  V  Q  V  S  N  C  T  142
 601 GTCCTGGGATGATATCCAGTGTGTTGAAGAATTTGGTGCCAATGCCACTGTGGAAACCCC  660
 143  S  W  D  D  I  Q  C  V  E  E  F  G  A  N  A  T  V  E  T  P  162
 661 AGCTGCTGAAGAGACAATGAACACCAGCCCGGGGACTCCTGCCCCAGCTGCTGAAGAGAC  720
 163  A  A  E  E  T  M  N  T  S  P  G  T  P  A  P  A  A  E  E  T  182
 721 AATGAACACCAGCCCAGGGACTCCTGCCCCAGCTGCTGAAGAGACAATGACCACCAGCCC  780
 183  M  N  T  S  P  G  T  P  A  P  A  A  E  E  T  M  T  T  S  P  202
 781 GGGGACTCCTGCCCCAGCTGCTGAAGAGACAATGACCACCAGCCCGGGGACTCCTGCCCC  840
 203  G  T  P  A  P  A  A  E  E  T  M  T  T  S  P  G  T  P  A  P  222
 841 AGCTGCTGAAGAGACAATGACCACCAGCCCGGGGACTCCTGCCTCTTCTCATTACCTCTC  900
 223  A  A  E  E  T  M  T  T  S  P  G  T  P  A  P  S  S  H  Y  L  S  242
 901 ATGCACCATCGTAGGGGATCATAGTTCTAATTGTGCTTCTGATTGTGTTTGTTGAAAGAC  960
 243  C  T  I  V  G  I  I  V  L  I  V  L  L  I  V  F  V  *  262
 961 TTCACTGTGGAAGAAATTCCTTCCTTACCTGAAAGGTTCACGTTACGCGCTGGCTGAAGG 1020
1021 CGGGGGGCGCTGGACACTCTCTGCCCTGCCTCCCTCTGCTGTGTTCCCACAGACAGAAAC 1080
1081 GCCTGCCCCTGCCCCAAGTCCCTGGTGTCTCCAGCCTGGCTCTATCTTCCTCCTTGTGATC 1140
1141 GTCCCATCCCACATCCCGTGCACCCCCCAGGACCCTGGTCTCATCAGTCCCTCTCCTGG 1200
1201 AGCTGGGGGTCCACACATCTCCCAGCCAAGTCCAAGAGGGCAGGGCCAGTTCCTCCCATC 1260
1261 TTCAGGCCCAGCCAGGCAGGGGGCAGTCGGCTCCTCAACTGGGTGACAAGGGTGAGGATG 1320
1321 AGAAGTGGTCACGGGATTTATTCAGCCTTGGTCAGAGCAGAAAAA              1365
```

FIG. 6

```
   1 CCAGCCTCAAACTGCAGTCCGGCGCCGCGGGGCAGGACAAGGGGAAGGAATAAACACGTT   60
  61 TGGTGAGAGCCATGGCACTCAAGGTCCTACCTCTACACAGGACGGTGCTCTTCGCTGCCA  120
  -2           M  A  L  K  V  L  P  L  H  R  T  V  L  F  A  A  I
 121 TTCTCTTCCTACTCCACCTGGCATGTAAAGTGAGTTGCGAAACCGGAGATTGCAGGCAGC   17
  18  F  L  L  L  H  L  A  C  K  V  S  C  E  T  G  D  C  R  Q  Q
 181 AGGAATTCAAGGATCGATCTGGAAACTGTGTCCTGTCCAAACAGTGCGGACCTGGCATGG  180
  38  E  F  K  D  R  S  G  N  C  V  L  C  K  Q  C  G  P  G  M  E   37
 241 AGTTGTCCAAGGAATGTGGCTTCGGCTATGGGGAGGATGCACAGTGTGTGCCCTGCAGGC  240
  58  L  S  K  E  C  G  F  G  Y  G  E  D  A  Q  C  V  P  C  R  P   57
 301 CGCACCGGTTCAAGGAAGACTGGGGTTTCCAGAAGTGTAAGCCATGTGCGGACTGTGCGC  300
  78  H  R  F  K  E  D  W  G  F  Q  K  C  K  P  C  A  D  C  A  L   77
 361 TGGTGAACCGCTTTCAGAGGGCCAACTGCTCACACACCAGTGATGCTGTCTGCGGGGACT  360
  98  V  N  R  F  Q  R  A  N  C  S  H  T  S  D  A  V  C  G  D  L   97
 421 GCCTGCCAGGATTTTACCGGAAGACCAAACTGGTTGGTTTTCAAGACATGGAGTGTGTGC  420
 118  L  P  G  F  Y  R  K  T  K  L  V  G  F  Q  D  M  E  C  V  P  117
 481 CCTGCCGGAGACCCACCTCCTCCCTACGAACCACACTGTACCAGCAAGGTGAACCTTGTGA  480
 138  C  G  D  P  P  P  P  Y  E  P  H  C  T  S  K  V  N  L  V  K  137
 541 AGATCTCCTCCACCGTCTCCAGCCCTCGGGACACGGCGCTGGCTGCCGTCATCTGCAGTG  540
 158  I  S  S  T  V  S  S  P  R  D  T  A  L  A  A  V  I  C  S  A  157
 601 CTCTGGCCACGGTGCTGCTCGCCCTGCTCATCCTGTGTGTCATCTACTGCAAGAGGCAGT  600
 178  L  A  T  V  L  L  A  L  L  I  L  C  V  I  Y  C  K  R  Q  F  177
 661 TCATGGAGAAGAAACCCAGCTGGTCTCTGCGGTCACAGGACATTCAGTACAATGGCTCTG  660
 198  M  E  K  K  P  S  W  S  L  R  S  Q  D  I  Q  Y  N  G  S  E  197
 721 AGCTGTCATGCTTTGACCAGCCTCGGCTCCGCCACTGTGCCCATAGAGCTGCTGTCAGT  720
 218  L  S  C  F  D  Q  P  R  L  R  H  C  A  H  R  A  C  C  Q  Y  217
 781 ATCACCGGGACTCAGCCCCAATGTATGGGCCTGTTCACCTGATTCCGTCCTTGTGCTGTG  780
 238  H  R  D  S  A  P  M  Y  G  P  V  H  L  I  P  S  L  C  C  E  237
 841 AAGAGGCCCGCAGCTCTGCCCGAGCTGTGCTTGGCTGTGGGCTGCGTTCTCCCACTACCC  840
 258  E  A  R  S  S  A  R  A  V  L  G  C  G  L  R  S  P  T  T  L  257
 901 TCCAGGAGAGAAACCCGGCTTCTGTGGGGAACACGATGCCAGCCTTCTTTGGGTCTGTTT  900
 278  Q  E  R  N  P  A  S  V  G  N  T  M  P  A  F  F  G  S  V  S  277
 961 CCCGTTCCATCTGCGCGGAGTTTTCTGATGCCTGGCCTCTGATGCAGAATCCTCTGGGCG  960
 298  R  S  I  C  A  E  F  S  D  A  W  P  L  M  Q  N  P  L  G  G  297
1021 GTGACAGCTCTCTCTGTGACTCTTATCGTGAACTCACTGGAGAAGATACCAATTCCCTCA 1020
 318  D  S  S  L  C  D  S  Y  P  E  L  T  G  E  D  T  N  S  L  N  317
1081 ATCCCGAAAACGAAAGCACAGCATCTCTGGATTCCAGTGGCGGCCAGGATCTGGCTGGGA 1080
 338  P  E  N  E  S  T  A  S  L  D  S  S  G  G  Q  D  L  A  G  T  337
1141 CAGCTGCTCTAGAGTCTTCTGGGAATGTTTCAGAATCTACTGCTCACCTAGACATGGTG 1140
 358  A  A  L  E  S  S  G  N  V  S  E  S  T  D  S  P  R  H  G  D  357
1201 ACACTGGTACAGTCTGGGAGCAGACGCTAGCTCAGGATGCTCAAAGGACTCCAAGCCAAG 1200
 378  T  G  T  V  W  E  Q  T  L  A  Q  D  A  Q  R  T  P  S  Q  G  377
1261 GAGGCTGGGAAGACAGGGAAAACCTGAATCTAGCCATGCCCACAGCCTTCCAGGATGCCT 1260
 398  G  W  E  D  R  E  N  L  N  L  A  M  P  T  A  F  Q  D  A  *  397
1321 GAAGGCCATCTTCCTGACGTGGAGGTGTGGGTCTGGACACGCCTGTGATGAGGCCTACAG 1320
                                                                  417
1381 ACTGAGCAGTCTTGGTGTCTGGAAGCAAAAATAAATCTGAACCAAACTGACAACATTTCC 1380
1441 ATCCTTTCAGCCACTAGCTACTGAGCCAGACCAGCTGTAAGCTGAAACCCAGCAAGAAG 1440
1501 CAAGGAGAGACTTGACTGTAGGCAGGACCCTTGGGACATGTGCTTCTTCCCTAAGCGAAAACC 1500
1561 TTAGCTGGGGCCAATTTGAAGGACCCATGGGTGGAATGTGCTGCCTGTGAACTTGTGGC 1560
1621 ACAGCAGGACCCAGCCTGGCTCCTTCTTATGTCCACGGTGAATGTGGTTTCACAAGAC 1620
                                                                 1678
```

FIG. 7A

```
  1  CCAGCCTCAAACTGCAGTCCGGCGCCGCGGGGCAGGACAAGGGGAAGGAATAAACACGTT  60
 61  TGGTGAGAGCCATGGCACTCAAGGTCCTACCTCTACACAGGGGGAAGGAATAAACACGTT 120
 -2             M  A  L  K  V  L  P  L  H  R  I  V  L  F  A  A  I  17
121  TTCTCTTCCTACTCCACCTGGCATGTAAAGTGAGTTGCGAAACCGGAGATTGCAGGCAGC 180
 18   L  F  L  L  H  L  A  C  K  V  S  C  E  T  G  D  C  R  Q  Q  37
181  AGGAATTCAAGGATCGATCTGGAAACTGTGTCCTCTGCAAACAGTGCGGACCTGGCATGG 240
 37      E  F  K  D  R  S  G  N  C  V  L  C  K  Q  C  G  P  G  M  E  57
241  AGTTGTCCAAGGAATGTGGCTTCGGCTATGGGGAGGATGCACAGTGTGTGCCCTGCAGGC 300
 58   L  S  K  E  C  G  F  G  Y  G  E  D  A  Q  C  V  P  C  R  P  77
301  CGCACCGGTTCAAGGAAGACTGGGGTTTCCAGAAGTGTAAGCCATGTGCGGACTGTGCGC 360
 78   H  R  F  K  E  D  Q  G  F  Q  K  C  K  P  C  A  D  C  A  L  97
361  TGGTGAACCGCTTTCAGAGGGCCAACTGCTCACACACCAGTGATGCTGTCTGCGGGGACT 420
 98   V  N  R  F  Q  R  A  N  C  S  H  T  S  D  A  V  C  G  D  C  117
421  GCCTGCCAGGATTTTTACCGGAAGACCAAACTGGTTGGTTTTCAAGACATGGAGTGTGTG 480
118   L  P  G  F  Y  R  K  T  K  L  V  G  F  Q  D  M  E  C  V  P  137
481  CCTGCGGAGACCCACCTCCTCCCTACGAACCACACTGTACCAGCAAGGTGAACCTTGTGA 540
138   C  G  D  P  P  P  P  Y  E  P  H  C  T  S  K  V  N  L  V  K  157
541  AGATCTCCTCCACCGTCTCCAGCCCTCGGGACACGGCGCTGGCTGCCGTCATCTGCAGTG 600
158   I  S  S  T  V  S  S  P  R  D  T  A  L  A  A  V  I  C  S  A  177
601  CTCTGGCCACGGTGCTGCTCGCCCTGCTCATCCTGTGTGTCATCTACTGCAAGAGGCAGT 660
178   L  A  T  V  L  L  A  L  L  I  L  C  V  I  Y  C  K  R  Q  F  197
661  TCATGGAGAAGAAACCCAGCTGTAAGCTCCCATCCCTCTGTCTCACTGTGAAGTGAGCTT 720
198   M  E  K  K  P  S  C  K  L  P  S  L  C  L  T  V  K  *  217
721  GTTAGCATTGTCACCCAAGAGTTCTCAAGACACCTGGCTGAGACCTAAGACCTTTAGAGC 780
781  ATCAACAGCTACTTAGAATACAAGATGCAGGAAAACGAGCCTCTTCAGGAATCTCAGGGC 840
841  CTCCTAGGGATGCTGGCAAGGCTGTGATGTCTCAAGGCTACCAGGA               886
```

FIG. 7B

```
   1  GGACCTGCAGCCTCCCAGGTGGCTGGGAAGAACTCTCCAACAATAAATACATTTGATAAG   60
  61  AAAGATGGCTTTAAAAGTGCTACTAGAACAAgAGAAAACGTTTTTCACTCTTTTAGTATT  120
   0        M  A  K  V  L  L  F  Q  E  K  I  F  F  T  L  L  V  I
 121  ACTAGGCTATTTGTCATGTAAAGTGACTTGTGAATCAGGAGACTGTAGACAGCAAGAATT  180
  20    L  G  Y  I  S  C  K  V  T  C  E  S  G  D  C  R  Q  Q  E  F
 181  CAGGGATCGGTCTGGAAACTGTGTTCCCTGCAACCAGTGTGGGCCAGGCATGGAGTTGTC  240
  40    R  D  R  S  G  N  C  V  P  C  N  Q  C  G  P  G  M  E  L  S
 241  TAAGGAATGTGGCTTCGGCTATGGGGAGGATGCACAGTGTGTGGCGTGCCGGCTGCACAG  300
  60    K  E  C  G  F  G  Y  G  E  D  A  Q  C  V  A  C  R  L  H  R
 301  GTTCAAGGAGGACTGGGGCTTCCAGAAATGCAAGCCCTGTCTGGACTGCGCAGTGGTGAA  360
  80    F  K  E  D  W  G  F  Q  K  C  K  P  C  L  D  C  A  V  V  N
 361  CCGCTTTCAGAAGGCAAATTGTTCAGCCACCAGTGATGCCATCTGCGGGGACTGCTTGCC  420
 100    R  F  Q  K  A  N  C  S  A  T  S  D  A  I  C  G  D  C  L  P
 421  AGGATTTTATAGGAAGACGAAACTTGTCGGCTTTCAAGACATGGAGTGTGTGCCTTGTGG  480
 120    G  F  Y  R  K  T  K  L  V  G  F  Q  D  M  E  C  V  P  C  G
 481  AGACCCTCCTCCTTACGAACCGCACTGTGCCAGCAAGGTCAACCTCGTGAAGATCGC     540
 140    D  P  P  P  P  Y  E  P  H  C  A  S  K  V  N  L  V  K  I  A
 541  GTCCACGGCCTCCAGCCCACGGGACACGGCGCTGGCTGCCGTTATCTGCAGCGCTCTGGC  600
 160    S  T  A  S  S  P  R  D  T  A  L  A  A  V  I  C  S  A  L  A
 601  CACCGTCCTGCTGGCCCTGCTCATCCTCTGTGTCATCTATTGTAAGAGACAGTTTATGGA  660
 180    T  V  L  L  A  L  L  I  L  C  V  I  Y  C  K  R  Q  F  M  E
 661  AAAGAAACCCAGCTGGTCTCTGCGGTCACAGGACATTCAGTACAACGAgactGAGCTGTC  720
 200    K  K  P  S  W  S  L  R  S  Q  D  I  Q  Y  N  E  T  E  L  S
 721  GTGTTTTGACAGACCTCAGCTCCACGAATATGCCCACAGAGCCTGCTGCCAGTGCCGCCG  780
 220    C  F  D  R  P  Q  L  H  E  Y  A  H  R  A  C  C  Q  C  R  R
 781  TGACTCAGTGCAGACCTGCGGGCCGGTGCGCTTGCTCCCATCCATGTGCTGTGAGGAGGC  840
 240    D  S  V  Q  T  C  G  P  V  R  L  L  P  S  M  C  C  E  E  A
 841  CTGCAGCCCCAACCCGGCGACTCTTGGTTGTGGGGTGCATTCTGCAGCCAGTCTTCAGGC  900
 260    C  S  P  N  P  A  T  L  G  C  G  V  H  S  A  A  S  L  Q  A
 901  AAGAAACGCAGGCCCAGGGGGAGATGGTGCCGACTTTCTTCGGATCCCTCACGCAGTC    960
 280    R  N  A  G  P  A  G  E  M  V  P  T  F  F  G  S  L  T  Q  S
 961  CATCTGTGGCGAGTTTTCAGATGCCTGGCCTCTGATGCAGAATCCCATGGGTGGTGACAA 1020
 300    I  C  G  E  F  S  D  A  W  P  L  M  Q  N  P  M  G  G  D  N
1021  CATCTCTTTTTGTGACTCTTATCCTGAACTCACTGGAGAAGACATTCATTCTCTCAATCC 1080
 320    I  S  F  C  D  S  Y  P  E  L  T  G  E  D  I  H  S  L  N  P
1081  AGAACTTGAAAGCTCAACGTCTTTGGATTCAAATAGCAGTCAAGATTTGGTTGGTGGGGC 1140
 340    E  L  E  S  S  T  S  L  D  S  N  S  S  Q  D  L  V  G  G  A
1141  TGTTCCAGTCCAGTCTCATTCTGAAAACTTTACAGCAGCTACTGATTTATCTAGATATAA 1200
 360    V  P  V  Q  S  H  S  E  N  F  T  A  A  T  D  L  S  R  Y  N
1201  CAACACTGGTAGAAATCAGCATCAACTCAGGATGCACTAACTATGAGAAGCCAGCTAGA 1260
 380    N  T  L  V  E  S  A  S  T  Q  D  A  L  T  M  R  S  Q  L  D
1261  TCAGGAGAGTGGCGCTGTCATCCACCCAGCCACTCAGACGTCCCTCCAGGTAAGGCACG 1320
 400    Q  E  S  G  A  V  I  H  P  A  T  Q  T  S  L  Q  V  R  Q  R
1321  ACTGGGTTCCCTGTGAACACAGCACTGACTTACAGTAGATCAGAACTCTGTTCCCAGCAT 1380
 420    L  G  S  L  *
1381  AAGATTTGGGGGAACCTGGATGAGTTTTTTTTTTTGCATCTTTAATAATTTCTTATATGT 1440
1441  TGTAGAGTATGTTTTAAAATAAATTTCAAGTATTTTTTTTAAAAAACTTT            1489
```

FIG. 7C

```
1     TGGCCTCTGATGCAGAGAATCCTCTGGGTGGTGACAGCTCTCTTCTGTGACTCTTATCCTGAA   60
      W  P  L  M  Q  N  P  L  G  G  D  S  L  C  D  S  Y  P  E            20
61    CTCACTGGAGAAGATATCAATTCCCTCAATCCGAAAATGAAAGCTCAACATCTGTGGAT       120
      L  T  G  E  D  I  N  S  L  N  P  E  N  E  S  S  T  S  V  D         40
121   TCCAATGGTGGTCAGGATCTGGCTGGGGCAGCTGCTAAAGCAGATTTTCAA              180
      S  N  G  G  Q  D  L  A  G  A  A  A  P  D  S  S  G  D  F  Q         60
181   GAAAATACTGACTCACTAGATATGGTGACGCCGATACGGTCTGGGAGCAAACCCTAGCT      240
      E  N  T  D  S  P  R  Y  G  D  A  D  T  V  W  E  Q  T  L  A         80
241   CAGGATGCTCAAAGGACTCCAAGCACCAGCAGAGAGGGCTGGGAAGCCAGTGAAAACCTGAATCTA  300
      Q  D  A  Q  R  T  P  S  R  E  G  W  E  A  S  E  N  L  N  L         100
301   GCCACGTCCACAGCCTTCCAGGATGCCTGAAGGCCATCTTCCTGACATGGAGGTGTGGGT     360
      A  T  S  T  A  F  Q  D  A  *                                       360
361   CTGGGCACGCCCTCTGTGAGTCTTACAGACTGAGCAGTCTTGACCTTCTACGGTGTCTGG      420
421   AAGGAAAAAATAAATCTGAAGCAAACTGGCAGCAACTGGGAAGGCACTCCATACTTTCAGCCACGAGCTTCT  480
481   GAGCCAGACCAGCTGTAGCTGAAGAGAGCCCGTAGTGGGAGTAGGCGGCC             540
541   TCGGGACCTGCACTCTTCCCTAAACAAGAACCTAGCTGGGGCCCAATTGAAGGACCAT        600
601   GATGGAATGTGCTGCCGTGTGAGATTGTGGGCACGGCAGGGCCCAGCCTGGCTCCTTCTTTA    660
661   CGTTCATGTGAATGTGGTTTCACAAGAACCAGAGTATAAACTTTCATAGACTTCTCTTT       720
721   TAAACATTACCCACTACCCTGGGAAAAAAAGCCTTTCTAATCANCAAAATNTTCTCCATTG      780
781   GTTTNAAGGGNCTNCTTACCCCCCGAATTGTTATTTNGGTGAAATTTAANTGGCCT         840
841   GCCCCTTATNCCCGGGTCTTNAAAGGTGGTTTTCNCNCCCTTNACNGGAA             900
901   AAGAGCCGGGATTAAAAGGGAAAGGGCTCCCTTTAATTTNCCCTNNTCCAATTTTCC        960
961   GGGAAGGAAGATTTTTAACCNTCCCCGTAATTTGGAANCTTTTTTTATTCCGC            1020
1081  TNAAACCTGNNTTTTAACCNTCCCCGTAATTTGGAANCTTTTTTTATTCCGC            1133
```

FIG. 7D

Nucleotide and amino acid sequence of mAPO4-γ

```
1    GCGTTTGGCGCGGAAGTGCTACCAAGCTGCGGAAAGCGTGAGTCTGGAGCACAGCACTGG                60
61   CGAGTAGCAGGAATAAACACGTTTGTGAGAGCCATGGCCATGCACTCAAGGTCCTACCTCTACA          120
-10                                              M  A  L  K  V  L  P  L  H        9
121  CAGGACGGTGCTCTTCGCTGCCATTCTCTTCCTACTTCCACCTGCCATGTAAAGTGAGTTG            180
10   R  T  V  L  F  A  A  I  L  F  L  L  H  L  A  C  K  V  S  C                 29
181  CGAAACCGGAGATTGCAGGCAGCAGGAATTCAAGGATCTGGAAACTGTGTCCTCTG               240
30   E  T  G  D  C  R  Q  Q  E  F  K  D  R  S  G  N  C  V  L  C                 49
241  CAAACAGTGCGGACCTGGCATGGAGTGTCCAAGAAGTGGCTTCGGCTTCGGGGGAGGA             300
50   K  Q  C  G  P  G  M  E  L  S  K  E  C  G  F  G  Y  G  E  D               69
301  TGCACAGTGTGTGCCCTGCAGGCCGCACCGGTTCAAGGAAGACTGGGGTTTCCAGAAGTG           360
70   A  Q  C  V  P  C  R  P  H  R  F  K  E  D  W  G  F  Q  K  C               89
361  TAAGCCATGTGCCGACTGTGCGCTGGTGAACCGCTTTCAGAGGGCCCAACTGCTCACACAC         420
90   K  P  C  A  D  C  A  L  V  N  R  F  Q  R  A  N  C  S  H  T               109
421  CAGTGATGCTGTCTGCGGGACTGGTGTGCCCAGGATTTTACCGGAAGACCAAACTGGTTGG         480
110  S  D  A  V  C  G  D  C  L  P  G  F  Y  R  K  T  K  L  V  G               129
481  TTTTCAAGACATGGAGTGTGTGCCCTGCGGAGACCCACCTCCTCCTACGAACCACACTG           540
130  F  Q  D  M  E  C  V  P  C  G  D  P  P  P  P  Y  E  P  H  C               149
541  TGAGTGATGTGCCAAGTGCAGCAGACCTTTAAAAAAAGAAAAAAAAAA                        591
150  E  *                                                                     166
```

FIG. 7E

Nucleotide and amino acid sequence of mAPO4-β

```
1    CCACGCGTCCGATCCTGAACTCACTGGAGAAGATACCAATCCCTCAATCCCGAAAACGA    60
1      H   A   S   D   P   E   L   T   G   E   D   T   N   S   L   N   P   E   N   E      20
61   AAGCGGCAGCATCTCTGGATTCCAGTGGCGCCAGGATCTGGCTGGGACAGCTGCTCTAGA   120
21     S   A   A   S   L   D   S   S   G   G   Q   D   L   A   G   T   A   A   L   E      40
121  GTCTTCTGGGAATGTTTCAGAATCTACTGACTCACCTAGACATGGTGACACTGTACAGT   180
41     S   S   G   N   V   S   E   S   T   D   S   P   R   H   G   D   T   G   T   V      60
181  CTGGGAGCAGACGCTAGCTCAGGATGCTCAAAGAGACTCCAAGCCCAAGGAGGCTGGGAAGA   240
61     W   E   Q   T   L   A   Q   D   A   Q   R   T   P   S   Q   G   G   W   E   D      80
241  CAGGGAAAACCTGAATCTAGCCATGCCCCACAGCCTTCCAGGATGCCTGAAGGCCATCTTC   300
81     R   E   N   L   A   H   P   T   A   F   Q   D   A   *                          95
301  CTGACGTGGGAGGTGTGGGTCTGGACAAGCCTGTGATGAGCCTACAGACTGAGCAGTCTT   360
361  GGTGTCTGGAAGCAAAAATAAATCTGAACCAAACTG                            396
```

FIG. 8

PARTIAL NUCLEOTIDE AND AMINO ACIDS SEQUENCE OF hAPO6

```
  1   TGGAGCGCTGCCGCTACTGCAACGTCCTCTGCGGGAGCGTGAGGAGGAGGACGGGCTT       60
  1    E  R  C  R  Y  C  N  V  L  C  G  G  E  R  E  E  E  A  R  A  C    20
 61   GCCACGCCACCCACAACCGTGCCTGCCGCCGCCCGCACCGGCCTTCTTCGGCGCACGCTGGTT  120
 21    H  A  T  H  N  R  A  C  R  C  R  T  G  F  F  A  H  A  G  F       40
121   TCTGCTTGAGCAGCACGCATCGTCCACCTGGTGCCGGCGTGATTGCCCCGGCACCCCA      180
 41    C  L  E  H  A  S  C  P  P  G  A  G  V  I  A  P  G  T  P  S       60
181   GCCAGAACACGCAGTGCCAGCCCGTGCCCCCAGGCACCTTCTCAGCCAGCTCCAGCT      240
 61    Q  N  T  Q  C  Q  P  P  G  T  F  S  A  S  S  S  S             80
241   CAGAGCAGTGCCAGCCCCACCGCCCAACTGCACGCCCCTGGCCCTCAATGTGCCAG       300
 81    E  Q  C  Q  P  H  R  N  C  T  A  L  G  L  A  L  N  V  P  G     100
301   GCTCTYTCCTCCATGACACCCCTGTGCACCAGCTGCACTGGCTTCCCCCCTCAGCCACCAGGG  360
101    S  S  S  H  D  T  L  C  T  S  C  T  G  F  P  L  S  T  R  V     120
361   TACCAGGAGCTGAGGAGTTGAGCGTGCCGCATCGACTTGTGGCTTCCAGGACATCT       420
121    P  G  A  E  E  C  E  R  A  V  I  D  F  V  A  F  Q  D  I  S     140
421   CCATCAAGAGGCTGCAGCGGCTGCAGGCCCTCGAGGCCCCGGAGGGCTGGGGTCCGA     480
141    I  K  R  L  Q  R  L  Q  A  L  E  A  P  E  G  W  G  P  T       160
481   CACCAAGGGCGGCGGCGCCGGGGCCTTGCAGCTGAAGCTGCGGCGGCGTCACGGAGCTCC   540
161    P  R  A  G  R  A  A  L  Q  L  K  L  R  R  R  L  T  E  L  L     180
541   TGGGGGGCGCAGGACGGGGGCGCTGCTGGTGCGTCTGCTGCAGGCgctGCGCGTGGCCAGGA   600
181    G  A  Q  D  G  A  L  L  V  R  L  L  Q  A  L  R  V  A  R  M     200
601   TGCCCGGGCTGGAGCGGAGCGTCCGGTGAGCGGCTTCTCCCCGTGCACTGATCCTGGCCCC   660
201    P  G  L  E  R  S  V  R  E  R  F  L  P  V  H  *               220
661   CTCTTATTTATTCTACATCCTTGGCACCCACTGCACTGAAAGAGGCCTTTTTTTAAAT     720
721   AGAGAAATGAGGTTTCTTAAAAAAAAAAAAAAAAAAAAAAAAAA   767
```

FIG. 9

FIG. IOA

FIG. IOB

118 hLT-α
143 hTNF-alpha
200 hFasL
144 hLT-β
180 hCD40L
193 hTRAIL
131 hTNRL3
134 mTNRL3
174 hTNRL1-β
165 mTNRL1-β
144 hTNRL1-α
209 mTNRL1-α

186 hLT-α
213 hTNF-alpha
262 hFasL
224 hLT-β
242 hCD40L
261 hTRAIL
188 hTNRL3
191 mTNRL3
229 hTNRL1-β
220 mTNRL1-β
203 hTNRL1-α
268 mTNRL1-α

NUCLEOTIDE AND AMINO ACID SEQUENCE OF FULL-LENGTH HUMAN TNRL-1 (TNRL-alpha)

```
   1  GAAAGGCAGAAAGGAGAGAAAATTCAGGATAACTCTCCTGAGGGGTGAGCCAAGCCCTGCCA          60
  61  TGTAGTGCACGCAGGACATCAACAACAACAGATAACAGAAAATGATCCATTCCCTGTVGGT         120
 121  CACTATTCTAAAGGCCCCAACCTTCAAAGTTCAAGTAGTGATATGGACTCCACAGAA            180
 -13                                        M  D  D  S  T  E             6
 181  AGGGAGCAGTCACGCCTTACTTCTTGCCTTAAGAAAAAGAAGAAGAAACTGAAGGAG           240
   7   R  E  Q  S  R  L  T  S  C  L  K  K  R  E  E  M  K  L  K  E         26
 241  TGTGTTTCCATCCTCCCAGGAAGAAAGCCCTCTGTCGGATCCTCCAAAGACGGAAAG           300
  27   C  V  S  I  L  P  R  K  E  S  P  S  V  R  S  S  K  D  G  K         46
 301  CTGCTGGCTGCAACCTTGCTGCTGGCACTCGCTGTCTTGTGCCTCACGGTGGTGTCTTTC        360
  47   L  L  A  A  T  L  L  A  L  L  S  C  C  L  T  V  V  S  F           66
 361  TACCAGGTGGCGGCCCCTGCCAGCCTGCGGGCAGAGCTGCAGGGCCAC                  420
  67   Y  Q  V  A  A  L  Q  G  D  L  A  S  L  R  A  E  L  Q  G  H        86
 421  CACGCGGAGAAGCTGCCAGCAGGAGCAGGAGCCCCCAAGGCCGGCCTGGAGGAAGCTCCA       480
  87   H  R  E  K  L  P  A  G  A  G  A  P  K  A  G  L  E  E  A  P        106
 481  GCTGTCACCGCGGGACTGAAAATCTTTGAACCACCAGCTCCAGGAGGCAACTCCAGT          540
 107   A  V  T  A  G  L  K  I  F  E  P  P  A  P  G  E  G  N  S           126
 541  CAGAACAGCAGAAATAAGCGTGCCGTTCAGGGTCAGAAGAAACAGTCACTCAAGACTGC        600
 127   Q  N  S  R  N  K  R  A  V  Q  G  P  E  E  T  V  T  Q  D  C        145
 601  TTGCAACTGATTGCAGACAGTGAAACACCAACTATACAAAAGGATCTTACACATTTGTT        660
 147   L  Q  L  I  A  D  S  E  T  P  T  I  Q  K  G  S  Y  T  F  V        166
 661  CCATGGCTTCTCAGCTTTAAAGGGGAGTGCCCTAGAGAAAAAGAGAATAAATATTG           720
 167   P  W  L  L  S  F  K  R  G  S  A  L  E  E  K  E  N  K  I  L        186
 721  GTCAAGAAACTGGTTACTTTTTTATATATGGTCAGGTTTTATATACTGATAAGACCTAC       780
 187   V  K  E  T  G  Y  F  F  I  Y  G  Q  V  L  Y  T  D  K  T  Y        206
 781  GCCATGGGACATCTAATTCAGAGAAGAAGGTCCATGTCTTTGGGGATGAATTGAGTCTG        840
 207   A  H  G  H  L  I  Q  R  R  K  V  H  V  F  G  D  E  L  S  L        226
 841  GTGACTTTGTTTCGATGTATTCAAAATATGCCTGAAACACTACCCAATAATTCCTGCTAT      900
 227   V  T  L  F  R  C  I  Q  N  M  P  E  T  L  P  N  N  S  C  Y        246
 901  TCAGCTGGCATTGCAAACTGCAAGGAAGGAGATGAACTCCAACTTGCAATACCAAGAGAA      960
 247   S  A  G  I  A  K  L  E  E  G  D  E  L  Q  L  A  I  P  R  E        266
 961  AATGCACAAATATCACTGGATGGAGATGTCACATTTTTTGGTGCATTGAAACTGCTGTGA    1020
 267   N  A  Q  I  S  L  D  G  D  V  T  F  F  G  A  L  K  L  L  *        286
1021  CCTACTTACACCATGTCGTAGCTATTTCCTCCCTTTCTCCGTACCTCTAAGAAGAAAG      1080
1081  AATCTAACTGAA  1092
```

FIG. I IA

Nucleotide and amino acid sequence of mTNRL1-α

```
   1 CAGGAGAGCGCTCCTGGGGGAACCCAGCCCTGCCATGCTCTGAGGGCAGTCTCCCAGGAC   60
  61 ACAGATGACAGGAAATGACCCACCCCTGTGGTCACTTACTCCAAAGGCCTAGACCTTCAA  120
 121 AGTGCTCCTCGTGGAATGGATGAGTCTGCAAAGACCCTGCCACCACCGGTGCCTCTGTTTT  180
  -4                 M   D   E   S   A   K   T   L   P   P   P   C   L   C   F
 181 TGCTCCGAGAAAGGAGAAGATATGAAAGTGGGATATGATCCCATCACTCCGCAGAAAGAG   240
  16  C   S   E   K   G   E   D   M   K   V   G   Y   D   P   I   T   P   Q   K   E
 241 GAGGGTGCCTGGTTTGGGATCTGCAGGGATGGAAGGCTGCTGGCTGCTACCCTCCTGCTG   300
  36  E   G   A   W   F   G   I   C   R   D   G   R   L   L   A   A   T   L   L   L
 301 GCCCTGTTGTCCAGCAGTTTCACAGCGATGTCCTTGTACCAGTTGGCTGCCTTGCAAGCA   360
  56  A   L   L   S   S   S   F   T   A   M   S   L   Y   Q   L   A   A   L   Q   A
 361 GACCTGATGAACCTGCGCATGGAGCTGCAGAGCTACCGAGGTTCAGCAACACCAGCCGCC   420
  76  D   L   M   N   L   R   M   E   L   Q   S   Y   R   G   S   A   T   P   A   A
 421 GCGGGTGCTCCAGAGTTGACCGCTGGAGTCAAACTCCTGACACCGGCAGCTCCTCGACCC   480
  96  A   G   A   P   E   L   T   A   G   V   K   L   L   T   P   A   A   P   R   P
 481 CACAACTCCAGCCGCGGCCACAGGAACAGACGCGCTTTCCAGGGACCAGAGGAAACAGAA   540
 116  H   N   S   S   R   G   H   R   N   R   R   A   F   Q   G   P   E   E   T   E
 541 CAAGATGTAGACCTCTCAGCTCCTCCTGCACCATGCCTGCCTGGATGCCGCCATTCTCAA   600
 136  Q   D   V   D   L   S   A   P   P   A   P   C   L   P   G   C   R   H   S   Q
 601 CATGATGATAATGGAATGAACCTCAGAAACAGAACTTACACATTTGTTCCATGGCTTCTC   660
 156  H   D   D   N   G   M   N   L   R   N   R   T   Y   T   F   V   P   W   L   L
 661 AGCTTTAAAAGAGGAAATGCCTTGGAGGAGAAAGAGAACAAAATAGTGGTGAGGCAAACA   720
 176  S   F   K   R   G   N   A   L   E   E   K   E   N   K   I   V   V   R   Q   T
 721 GGCTATTTCTTCATCTACAGCCAGGTTCTATACACGGACCCCATCTTTGCTATGGGTCAT   780
 196  G   Y   F   F   I   Y   S   Q   V   L   Y   T   D   P   I   F   A   M   G   H
 781 GTCATCCAGAGGAAGAAAGTACACGTCTTTGGGGACGAGCTGAGCCTGGTGACCCTGTTC   840
 216  V   I   Q   R   K   K   V   H   V   F   G   D   E   L   S   L   V   T   L   F
 841 CGATGTATTCAGAATATGCCCAAAACACTGCCCAACAATTCCTGCTACTCGGCTGGCATC   900
 236  R   C   I   Q   N   M   P   K   T   L   P   N   N   S   C   Y   S   A   G   I
 901 GCGAGGCTGGAAGAAGGAGATGAGATTCAGCTTGCAATTCCTCGGGAGAATGCACAGATT   960
 256  A   R   L   E   E   G   D   E   I   Q   L   A   I   P   R   E   N   A   Q   I
 961 TCACGCAACGGAGACGACACCTTCTTTGGTGCCCTAAAACTGCTGTAACTCACTTGCTGG  1020
 276  S   R   N   G   D   D   T   F   F   G   A   L   K   L   L   *
1021 AGTGCGTGATCCCCTTCCCTCGTCTTCTCTGTACCTCCGAGGGGAGAAACAGACGACTGGA  1080
 295
1081 AAAACTAAAAGATGGGGAAAGCCGTCAGCGAAAGTTTTCTCGTGACCCGTTGAATCTGAT  1140
1141 CCAAACCAGGAAATATAACAGACAGCCACAACCGAAGTGTGCCATGTGAGTTATGAGAAA  1200
1201 CGGAGCCCGCGCTCAGAAAGACCGGATGAGGAAGACCGTTTTCTCCAGTCCTTTGCCAAC  1260
1261 ACGCACCGCAACCTTGCTTTTTGCCTTGGGTGACACATGTTCAGAATGCAGGGAGATTTC  1320
1321 CTTGTTTTGCGATTTGCCATGAGAAGAGGGCCCACAACTGCAGGTCACTGAAGCATTCAC  1380
1381 GCTAAGTCTCAGGATTTACTCTCCCATCACATGCTAAGTACACACACGCTCTTTTCCAGG  1440
1441 TAATACTATGGGATACTATGGAAAGGTTGTTTGTTTTTAAATCTAGAAGTCTTGAACTGG  1500
1501 CAATAGACA   1509
```

FIG. 11B

```
   1 ACAACCTTCTTTCCCTTCTGCACCACTGCCGTACCCTACCCGCCCGCCACCTCCTTGC          60
  61 TACCCCACTCTTGAAACCACCAGCTGTTGGCAGGGTCCCCAGCCTCATCGCCAGCCTCATCTC   120
 -13                                     M  P  A  S  S  P
 121 CTTCTTGCTAGCCCCCAAAGGGCCTCCAGGCAACACATGGGGGGGCCCAGTCAGAGAGCCGG    180
   7  F  L  L  A  P  K  G  P  P  G  N  M  G  G  P  V  R  E  P  A
 181 CACTCTCAGTTGCCCTCTGGTTGAGTTGGGGGGCAGCTCTGGGGGCCGTGGCTTGTGCCA    240
  27  L  S  V  A  L  W  L  S  W  G  A  A  L  G  A  V  C  A  M
 241 TGGCTCTGCTGACCCAACAAACAGAGCTGCAGAGCCTCAGGAGAGAGGTGAGCCGGCTGC    300
  47  A  L  L  T  Q  Q  T  E  L  Q  S  L  R  R  E  V  S  R  L  Q
 301 AGGGGACAGGAGGCCCTCCCAGAATGGGGAAGGGTATCCCTGGCAGAGTCTCCCGGAGC    360
  67  Q  G  T  G  G  P  S  Q  N  G  E  G  Y  P  W  Q  S  L  P  E
 361 AGAGTTCCGATGCCCTGGAAGCCTGGGAGAGTGGGGAGAGATCCCGGAAAGGAGAGCAG    420
  87  Q  S  S  D  A  L  E  A  W  E  S  G  E  R  S  R  K  R  A  V
 421 TGCTCACCCAAAAACAGAAGAAGCAGCACTCTGTCTGCCACCTGGTTCCCATTAACGCCA    480
 107  L  T  Q  K  Q  K  K  Q  H  S  V  L  H  L  V  P  I  N  A  T
 481 CCTCCAAGGATGACTCCGATGTGACAGAGGTGGCAACAGCTCTTAGGCGTGGGA    540
 127  S  K  D  D  S  D  V  T  E  V  M  W  Q  P  A  L  R  R  G  R
 541 GAGGCCTACAGGGCCCAAGGGATATGGTGTCCGAATCCAGGATGCTGGAGTTTATCTGTGT    600
 147  G  L  Q  G  Q  G  Y  G  V  R  I  Q  D  A  G  V  Y  L  L  Y
 601 ATAGCCAGGTCCTGTTTCAAGACGTGACTTTCACCATGGGTGGTGTCTCTGAGAAG    660
 167  S  Q  V  L  F  Q  D  V  T  F  T  M  G  Q  V  V  S  R  E  G
 661 GCCAAGGAGGAGGCAGGAGACTCTATTCGCATGTATAAGAAGTATCCCTCCCACCGGACC    720
 187  Q  G  R  Q  E  T  L  F  R  C  I  R  S  M  P  S  H  P  D  R
 721 GGGCCTACAACAGCTGCTATAGCCGCAGGTGTCTTCCATTTACCAACGGGGATATTCTGA    780
 207  A  Y  N  S  C  Y  S  A  G  V  F  H  L  H  Q  G  D  I  L  S
 781 GTGTCATAATTCCCCGGCAAGGGCGAAACTTAACCTCTCTCCACATGGAACCTTCCTGG    840
 227  V  I  I  P  R  A  R  A  K  L  N  L  S  P  H  G  T  F  L  G
 841 GGTTTGTGAAACTGTGTTATTGTTATAAAAAGTGGCTCCCAGCTTGGAAGACCAGGGTGG    900
 247  F  V  K  L  *
 901 GTACATACTGGAGACAGCCAAGAGCTGAGTATATAAAGGAGAGGGATGTGCAGGAACAG    960
 961 AGGCGTCTTCCTGGGTTGGATGCCTTTTTGCTTTTTCCCTTTTCATTCCACCCCT   1020
1021 AGACTTTGATTTTACGGATATCTTGCTTCTGTTCCCCATGGAGCTCCGAATCTGCGTG   1080
1081 TGTGTAGATGAGGGGCGGCGGGGACGGGCGCCAGGCATTGTCCAGGACCTGGTCGGGGCCCAC   1140
1141 TGGAAGCATCCAGAACAGCACCACCATG 1168
```

FIG. 12A

FIG. 12B

FIG. 13A

```
  1  CTGGTCGTGGTCAGCCTGGGGGAGCTGGGCAAGCGCTGTCTGCCCAGGAGCCTTCTCAGGAG   60
  1   L  V  V  V  S  L  G  G  S  W  A  T  L  S  A  Q  E  P  S  Q  E     20

 61  GAGCTGACAGCAGAGGACCGCCGGGAGCCCCCTGAACTGAATCCCAGACAGAGGAAAGC     120
 21   E  L  T  A  E  D  R  R  E  P  P  E  L  N  P  Q  T  R  E  S        40

121  CAGGATGTGGTACCTTTCTTGGAACAACTAGTCCGGCCTCGGAGAAGTGCTCCTAAAGGC    180
 41   Q  D  V  V  P  F  L  E  Q  L  V  R  P  R  R  S  A  P  K  G         60

181  CGGAAGGCCGGGCCTGCCGAGCTATTGCAGCCCATTATGAGGTTCATCCTCGGCCAGGA     240
 61   R  K  A  R  A  G  L  P  E  L  L  Q  P  I  M  R  F  I  L  G  Q  G   80

241  CAGGATGGAGCACAAGCAGGTGTGGATGGGAGCAGTGAGTGGCTGGGAAGAGACCAAAATC    300
 81   Q  D  G  A  Q  A  G  V  D  G  S  S  E  W  E  E  T  K  I          100

301  AACAGCTCCAGCCCCTCTGCGCTACGACCGCCAGATTGGGGAATTTACAGTCATCAGGGCT    360
101   N  S  S  S  P  L  R  Y  D  R  Q  I  G  E  F  T  V  I  R  A       120

361  GGGCTCTACTACCTGTACTGTCAGGTGCACTTTGATGAGGGAAAGGCTACCTGAAG        420
121   G  L  Y  Y  L  Y  C  Q  V  H  F  D  E  G  K  A  V  L  K          140

421  CTGGACTTGCTGGTGAACGGTGTGCTGGCCCTGCGCCTGCTGCCAGGTGTCTGGGCGCTG    480
141   L  D  L  L  V  N  G  V  L  A  L  R  C  L  E  E  F  S  A  T       160

481  GCAGCAAGCTCTCCTCGGCCCCAGTCCGTTGTGTCGCCACCCTCCCCTGGGCTGTGGCCCCC   540
161   A  A  S  S  L  P  R  P  Q  S  R  L  L  A  L  L  L  A  A  P       180

541  CGGCCAGGGTCTTCCCTTCGGATCGCAGCCTTTCACTTAAGGCTGCCCCC            600
181   R  P  G  S  R  I  R  T  L  K  A  A  P                            200

601  TTCCTAACCTACTTTGGACTCTTTCAAGTTCACTGAGGGGCCTTGCTCTCCCAGATTCCT    660
201   F  L  T  Y  F  G  L  F  Q  V  H  *                               220

661  TAAACTTTCCCTGGCTCCAGGAGCATCACCACCTCCCTA    701
```
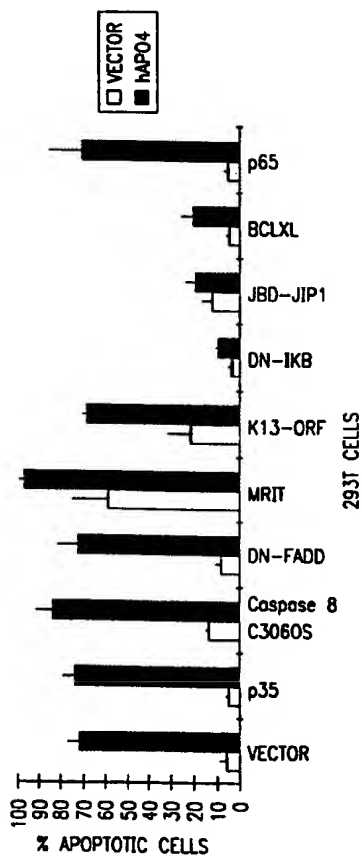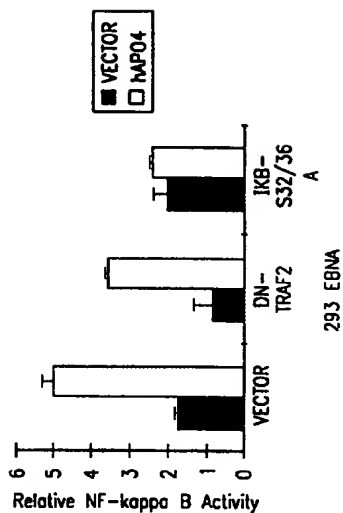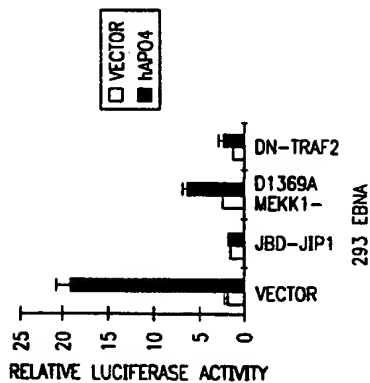
FIG. 13B

FIG. 14A

FIG. 14B

FIG. 14C